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<b>(54) Title:</b> DNA ENCODING TAURINE AND GABA TRANSPORTERS AND USES THEREOF  <b>(57) Abstract</b>  This invention provides isolated nucleic acid molecules, proteins, monoclonal antibodies, pharmaceutical compositions, transgenic animals, methods of treatment, methods of screening, and methods of diagnosis for both the GABA transporter and taurine transporter.		

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DNA ENCODING TAURINE AND GABA TRANSPORTERS AND USES  
THEREOF

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This application is a continuation-in-part of U.S. Serial No. 847,742, filed March 4, 1992 the contents of all of which are hereby incorporated by reference into the subject application.

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Background of the Invention

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Throughout this application various publications are referred to by partial citations within parenthesis. Full citations for these publications may be found at the end of the specification immediately preceding the claims. The disclosures of these publications, in their entireties, are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

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Chemical neurotransmission is a multi-step process which involves release of neurotransmitter from the presynaptic terminal, diffusion across the synaptic cleft, and binding to receptors resulting in an alteration in the electrical properties of the postsynaptic neuron. For most neurotransmitters, transmission is terminated by the rapid uptake of neurotransmitter via specific, high-affinity transporters located in the presynaptic terminal and/or surrounding glial cells (29). Since inhibition of uptake by pharmacologic agents increases the levels of neurotransmitter in the synapse, and thus enhances synaptic transmission, neurotransmitter transporters provide important targets for therapeutic intervention.

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The amino acid GABA is the major inhibitory neurotransmitter in the vertebrate central nervous system and is thought to serve as the neurotransmitter at approximately 40% of the synapses in the mammalian brain (13,28). GABAergic transmission is mediated by two classes of GABA receptors. The more prevalent is termed GABA<sub>A</sub>, which is a multi-subunit protein containing an intrinsic ligand-gated chloride channel in addition to binding sites for a variety of neuroactive drugs including benzodiazepines and barbiturates (35,73). In contrast, GABA<sub>B</sub> receptors couple to G-proteins and thereby activate potassium channels (2,35) and possibly alter levels of the second messenger cyclic AMP (35). Positive modulation of GABA<sub>A</sub> receptors by diazepam and related benzodiazepines has proven extremely useful in the treatment of generalized anxiety (77) and in certain forms of epilepsy (57).

Inhibition of GABA uptake provides a novel therapeutic approach to enhance inhibitory GABAergic transmission in the central nervous system (36,62). Considerable evidence indicates that GABA can be taken up by both neurons and glial cells, and that the transporters on the two cell types are pharmacologically distinct (15,36,62). A GABA transporter with neuronal-type pharmacology designated GAT-1 has previously been purified and cloned (21), but the molecular properties of other GABA transporters including glial transporter(s) have not yet been elucidated. We now report the cloning of two additional GABA transporters (GAT-2 and GAT-3) with distinct pharmacology and localization, revealing previously unsuspected heterogeneity in GABA transporters.



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Taurine (2-aminoethane sulfonic acid) is a sulfur-containing amino acid present in high concentrations in mammalian brain as well as various non-neural tissues. Many functions have been ascribed to taurine in both the nervous system and peripheral tissues. The best understood (and phylogenetically oldest) function of taurine is as an osmoregulator (26,75). Osmoregulation is essential to normal brain function and may also play a critical role in various pathophysiological states such as epilepsy, migraine, and ischemia. The primary mechanism by which neurons and glial cells regulate osmolarity is via the selective accumulation and release of taurine. Taurine influx is mediated via specific, high-affinity transporters which may contribute to efflux as well. Since taurine is slowly degraded, transport is an important means of regulating extracellular taurine levels.

Taurine is structurally related to the inhibitory amino acid  $\gamma$ -aminobutyric acid (GABA) and exerts inhibitory effects on the brain, suggesting a role as a neurotransmitter or neuromodulator. Taurine can be released from both neurons and glial cells by receptor-mediated mechanisms as well as in response to cell volume changes (64). Its effects in the CNS may be mediated by GABA<sub>A</sub> and GABA<sub>B</sub> receptors (34,56) and by specific taurine receptors (78). Additionally, taurine can also regulate calcium homeostasis in excitable tissues such as the brain and heart (26,41), via an intracellular site of action. Together, the inhibitory and osmoregulatory properties of taurine suggest that it acts as a cytoprotective agent in the brain. Depletion of taurine results in retinal degeneration in cats (70), supporting a role in neuronal survival.

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Although most animals possess the ability to synthesize taurine, many are unable to generate sufficient quantities and therefore rely on dietary sources. Taurine transport is thus critical to the maintenance of appropriate levels of taurine in the body. High-affinity, sodium-dependent taurine uptake has been observed in brain and various peripheral tissues (27,64), but little is known about the molecular properties of the taurine transporter(s). Cloning of the taurine transporter will not only help elucidate the function of this important neuro-active molecule, but may also provide important insight into novel therapeutic approaches to treat neurological disorders.

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cDNA clones (designated rB14b, rB8b, and rB16a) encoding transporters for two novel GABA transporters and a taurine transporter, respectively, have been isolated from rat brain, and their functional properties have been examined in mammalian cells. The transporters encoded by rB14b and rB8b display high-affinity for GABA ( $K_m=4\mu M$ ), and exhibit pharmacological properties distinct from the neuronal GABA transporter; the transporter encoded by rB16a displays high-affinity for taurine. All three are dependent on external sodium and chloride for transport activity. The nucleotide sequences of the three clones predict proteins of 602, 627, and 621 amino acids, respectively. Hydropathy analysis reveals stretches of hydrophobic amino acids suggestive of 12 transmembrane domains, similar to that proposed for other cloned neurotransmitter transporters. The cloning of two additional GABA transporters and a taurine transporter from rat brain reveals previously undescribed heterogeneity in inhibitory amino acid transporter genes.

The use of human gene products in the process of drug development offers significant advantages over those of other species, which may not exhibit the same pharmacological profiles. To facilitate this human-target based approach to drug design in the area of inhibitory amino acid transporters, we used the nucleotide sequences of the rat GAT-2 and GAT-3 cDNAs to clone the human homologue of each gene. cDNA clones (designated hHE7a, hS3a, hFB16a and hFB20a encoding the human homologue of the two novel GABA transporters GAT-2 and GAT-3 have been isolated.

Summary of the Invention

5 This invention provides an isolated nucleic acid molecule encoding a mammalian GABA transporter. In one embodiment of this invention, the nucleic acid molecule comprises a plasmid designated EVJB-rB14b (ATCC Accession No. ). In another embodiment of this invention, the nucleic acid molecule comprises a plasmid designated EVJB-rB8b (ATCC  
10 Accession No. ).

This invention also provides an isolated nucleic acid molecule encoding a mammalian taurine transporter. In one embodiment of this invention, the nucleic acid  
15 molecule comprises a plasmid designated EVJB-rB16a (ATCC Accession No. ).

This invention further provides isolated nucleic acid molecules encoding the human homologue of the mammalian  
20 GABA transporters. In one embodiment of this invention, the nucleic acid molecule comprises a plasmid designated pcEXV-hGAT-3 (ATCC Accession No. ). In another embodiment of this invention, the nucleic acid molecule comprises a plasmid designated pBluescript-hHE7a (ATCC  
25 Accession No. ). In another embodiment of this invention, the nucleic acid molecule comprises the plasmid pBluescript-hS3a (ATCC Accession No. ).

30 This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a mammalian GABA transporter. This invention  
35 also provides a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a

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sequence included within the sequence of a nucleic acid molecule encoding a mammalian taurine transporter. This invention also provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human GABA transporter. This invention also provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human taurine transporter.

This invention further provides an antisense oligonucleotide having a sequence capable of binding specifically to an mRNA molecule encoding a mammalian GABA transporter so as to prevent translation of the mRNA molecule. This invention also provides an antisense oligonucleotide having a sequence capable of binding specifically to an mRNA molecule encoding a mammalian taurine transporter so as to prevent translation of the mRNA molecule. This invention also provides an antisense oligonucleotide having a sequence capable of binding specifically to an mRNA molecule encoding a human GABA transporter so as to prevent translation of the mRNA molecule. This invention also provides an antisense oligonucleotide having a sequence capable of binding specifically to an mRNA molecule encoding a human taurine transporter so as to prevent translation of the mRNA molecule.

A monoclonal antibody directed to a mammalian GABA transporter is provided by this invention. A monoclonal antibody directed to a mammalian taurine transporter is also provided by this invention. A monoclonal antibody

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directed to a human GABA transporter is also provided by this invention. A monoclonal antibody directed to a human taurine transporter is also provided by this invention.

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This invention provides a pharmaceutical composition comprising an amount of a substance effective to alleviate the abnormalities resulting from overexpression of a mammalian GABA transporter and a pharmaceutically acceptable carrier as well as a pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of GABA transporter and a pharmaceutically acceptable carrier.

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A pharmaceutical composition comprising an amount of a substance effective to alleviate the abnormalities resulting from overexpression of a mammalian taurine transporter and a pharmaceutically acceptable carrier as well as a pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of a taurine transporter and a pharmaceutically acceptable carrier is also provided by this invention.

25

A pharmaceutical composition comprising an amount of a substance effective to alleviate the abnormalities resulting from overexpression of a human GABA transporter and a pharmaceutically acceptable carrier as well as a pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of a human GABA transporter and a pharmaceutically acceptable carrier is also provided by this invention.

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A pharmaceutical composition comprising an amount of a substance effective to alleviate the abnormalities resulting from overexpression of a human taurine transporter and a pharmaceutically acceptable carrier as well as a pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of a human taurine transporter and a pharmaceutically acceptable carrier is also provided by this invention.

This invention also provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a mammalian GABA transporter so positioned within such genome as to be transcribed into antisense mRNA complementary to mRNA encoding the GABA transporter and when hybridized to mRNA encoding the GABA transporter, the complementary mRNA reduces the translation of the mRNA encoding the GABA transporter.

This invention also provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a mammalian taurine transporter so positioned within such genome as to be transcribed into antisense mRNA complementary to mRNA encoding the taurine transporter and when hybridized to mRNA encoding the taurine transporter, the complementary mRNA reduces the translation of the mRNA encoding the taurine transporter.

This invention also provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a human GABA transporter so positioned within such genome as to be transcribed into antisense mRNA which is complementary to mRNA encoding the human GABA transporter and when hybridized to mRNA encoding the human GABA transporter,

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the antisense mRNA thereby reduces the translation of mRNA encoding the human GABA transporter.

5 This invention also provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a human taurine transporter so positioned within such genome as to be transcribed into antisense mRNA which is complementary to mRNA encoding the human taurine transporter and when hybridized to mRNA encoding the human taurine transporter, the antisense mRNA thereby reduces the translation of mRNA encoding the human taurine transporter.

15 This invention also provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a mammalian GABA transporter so positioned within such genome as to be transcribed into antisense mRNA which is complementary to mRNA encoding the transporter and when hybridized to mRNA encoding the transporter, the antisense mRNA thereby prevents the translation of mRNA encoding the transporter.

25 This invention also provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a mammalian taurine transporter so positioned within such genome as to be transcribed into antisense mRNA which is complementary to mRNA encoding the transporter and when hybridized to mRNA encoding the transporter, the antisense mRNA thereby prevents the translation of mRNA encoding the transporter.

35 This invention also provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a human GABA transporter so positioned within such genome as to be transcribed into antisense mRNA which is complementary to



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mRNA encoding the transporter and when hybridized to mRNA encoding the human GABA transporter, the antisense mRNA thereby prevents the translation of mRNA encoding the human GABA transporter.

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This invention also provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a human taurine transporter so positioned within such genome as to be transcribed into antisense mRNA which is complementary to mRNA encoding the human taurine transporter and when hybridized to mRNA encoding the human taurine transporter, the antisense mRNA thereby prevents the translation of mRNA encoding the human taurine transporter.

15

This invention provides a method of screening drugs to identify drugs which specifically interact with, and bind to, a mammalian GABA transporter on the surface of a cell which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding a mammalian GABA transporter, the protein encoded thereby is expressed on the cell surface, with a plurality of drugs, determining those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, a mammalian GABA transporter.

This invention provides a method of screening drugs to identify drugs which specifically interact with, and bind to, a mammalian taurine transporter on the surface of a cell which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding a mammalian taurine transporter, the protein encoded thereby is expressed on the cell surface, with a plurality of drugs, determining those drugs which bind to the mammalian cell,

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and thereby identifying drugs which specifically interact with, and bind to, a mammalian taurine transporter.

5 This invention provides a method of screening drugs to identify drugs which specifically interact with, and bind to, a human GABA transporter on the surface of a cell which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding a human GABA transporter, the protein encoded thereby is expressed on the cell  
10 surface, with a plurality of drugs, determining those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, a human GABA transporter.

15 This invention provides a method of screening drugs to identify drugs which specifically interact with, and bind to, a human taurine transporter on the surface of a cell which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding a human taurine  
20 transporter, the protein encoded thereby is expressed on the cell surface, with a plurality of drugs, determining those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, a human taurine transporter.

25 This invention also provides a method of determining the physiological effects of expressing varying levels of mammalian GABA transporters which comprises producing a transgenic nonhuman animal whose levels of mammalian GABA  
30 transporter expression are varied by use of an inducible promoter which regulates mammalian GABA transporter expression.

35 This invention also provides a method of determining the physiological effects of expressing varying levels of

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5 mammalian taurine transporters which comprises producing  
a transgenic nonhuman animal whose levels of mammalian  
taurine transporter expression are varied by use of an  
inducible promoter which regulates mammalian taurine  
transporter expression.

10 This invention also provides a method of determining the  
physiological effects of expressing varying levels of  
human GABA transporters which comprises producing a  
transgenic nonhuman animal whose levels of human GABA  
transporter expression are varied by use of an inducible  
promoter which regulates human GABA transporter  
expression.

15 This invention also provides a method of determining the  
physiological effects of expressing varying levels of  
human taurine transporters which comprises producing a  
transgenic nonhuman animal whose levels of human taurine  
transporter expression are varied by use of an inducible  
20 promoter which regulates human taurine transporter  
expression.

25 This invention further provides a method of determining  
the physiological effects of expressing varying levels of  
mammalian GABA transporters which comprises producing a  
panel of transgenic nonhuman animals each expressing a  
different amount of mammalian GABA transporter.

30 This invention further provides a method of determining  
the physiological effects of expressing varying levels of  
mammalian taurine transporters which comprises producing  
a panel of transgenic nonhuman animals each expressing a  
different amount of mammalian taurine transporter.

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This invention further provides a method of determining the physiological effects of expressing varying levels of human GABA transporters which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of human GABA transporter.

This invention further provides a method of determining the physiological effects of expressing varying levels of human taurine transporters which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of human taurine transporter.

This invention provides a method for diagnosing a predisposition to a disorder associated with the expression of a specific mammalian GABA transporter allele and a method for diagnosing a predisposition to a disorder associated with the expression of a specific mammalian taurine transporter allele which comprises:

- a.) obtaining DNA of subjects suffering from the disorder;
- b.) performing a restriction digest of the DNA with a panel of restriction enzymes;
- c.) electrophoretically separating the resulting DNA fragments on a sizing gel;
- d.) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a mammalian GABA or a mammalian taurine transporter and labelled with a detectable marker;
- e.) detecting labelled bands which have hybridized to the DNA encoding a mammalian GABA or taurine transporter labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder;
- f.) preparing DNA obtained for diagnosis by steps a-e; and
- g.) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether

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the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

5 This invention provides a method for diagnosing a predisposition to a disorder associated with the expression of a specific human GABA transporter allele or a specific human taurine transporter allele which comprises: a.) obtaining DNA of subjects suffering from  
10 the disorder; b.) performing a restriction digest of the DNA with a panel of restriction enzymes; c.) electrophoretically separating the resulting DNA fragments on a sizing gel; d.) contacting the resulting gel with a nucleic acid probe capable of specifically  
15 hybridizing to DNA encoding a human GABA or human taurine transporter and labelled with a detectable marker; e.) detecting labelled bands which have hybridized to the DNA encoding a human GABA or human taurine transporter labelled with a detectable marker to create a unique band  
20 pattern specific to the DNA of subjects suffering from the disorder; f.) preparing DNA obtained for diagnosis by steps a-e; and g.) comparing the unique band pattern specific to the DNA of subjects suffering from the  
25 disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

30 This invention provides a method for determining whether a substrate not known to be capable of binding to a mammalian transporter can bind to the mammalian GABA transporter which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding the GABA transporter with the substrate under conditions  
35 permitting binding of substrates known to bind to a

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transporter, detecting the presence of any of the substrate bound to the GABA transporter, and thereby determining whether the substrate binds to the GABA transporter.

5

This invention provides a method for determining whether a substrate not known to be capable of binding to a taurine transporter can bind to a taurine transporter which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding the taurine transporter with the substrate under conditions permitting binding of substrates known to bind to a transporter, detecting the presence of any of the substrate bound to the taurine transporter, and thereby determining whether the substrate binds to the taurine transporter.

10

15

This invention provides a method for determining whether a substrate not known to be capable of binding to a human GABA transporter can bind to a human GABA transporter which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding the human GABA transporter with the substrate under conditions permitting binding of substrates known to bind to a transporter, detecting the presence of any of the substrate bound to the human GABA transporter, and thereby determining whether the substrate binds to the human GABA transporter.

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25

This invention provides a method for determining whether a substrate not known to be capable of binding to a human taurine transporter can bind to a human taurine transporter which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding the human taurine transporter with the substrate under conditions permitting binding of substrates known to bind to a transporter, detecting the presence of any of the

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substrate bound to the human taurine transporter, and thereby determining whether the substrate binds to the human taurine transporter.

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Brief Description of the Figures

Figure 1. Nucleotide Sequence, Deduced Amino Acid Sequence and Putative Membrane Topology of Two Novel Mammalian GABA Transporters and a Novel Mammalian Taurine Transporter. A. Mammalian GABA transporter encoded by GAT-2 (rB14b) (Seq. I.D. Nos. 1 and 2). Nucleotides are presented in the 5' to 3' orientation and the coding region is numbered starting from the putative initiating methionine and ending in the termination codon. Deduced amino acid sequence by translation of a long open reading frame is shown. B. Mammalian GABA transporter encoded by GAT-3 (rB8b) (Seq. I.D. Nos. 3, and 4). Nucleotides are presented in the 5' to 3' orientation and the coding region is numbered starting from the putative initiating methionine and ending in the termination codon. Deduced amino acid sequence by translation of a long open reading frame is shown. C. Taurine transporter encoded by rB16a (Seq. I.D. Nos. 5 and 6). Nucleotides are presented in the 5' to 3' orientation and the coding region is numbered starting from the putative initiating methionine and ending in the termination codon. Deduced amino acid sequence by translation of a long open reading frame is shown. D. Deduced amino acid sequence and putative membrane topology of GABA transporter GAT-2 (rB14b). Deduced amino acid sequence by translation of a long open reading frame in rB14b is shown. Residues which are identical to those of GAT-3 (rB8b) are shaded. Membrane topology is modeled after that proposed for GAT-1 (21). E. Deduced amino acid sequence and putative membrane topology of taurine transporter (rB16a). Deduced amino acid sequence by translation of a long open reading frame in rB16a is shown. Membrane topology is modeled after that proposed for GAT-1 (21).

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Figure 2. Alignment of the novel GABA transporters with the rat neuronal GABA transporter, the betaine transporter, and the glycine transporter. The twelve putative  $\alpha$ -helical membrane spanning domains (I-XII) are bracketed. Residues identical to those of GAT-2 are shaded. GAT-2 is the GABA transporter encoded by rB14b; GAT-3 is the GABA transporter encoded by rB8b; GAT-1 is the rat neuronal GABA transporter (21), Betaine is the dog betaine transporter (79), and Glycine is the rat glycine transporter (68).

Figure 3. GABA transport by COS cells transfected with clone rB14b and rB8b. Non-transfected COS cells (control) or COS cells transfected with GAT-2 (panel A) or GAT-3 (panel B) were incubated for 10 minutes (37°C) with 50nM [ $^3$ H]GABA in either HBS (150mM NaCl) or in a similar solution in which Na<sup>+</sup> was replaced by equimolar Li<sup>+</sup> (Na<sup>+</sup>-free), or Cl<sup>-</sup> was replaced by acetate (in some experiments, calcium gluconate was used instead of calcium acetate; Cl<sup>-</sup>-free). Data show the specific uptake of GABA, expressed as pmoles/mg protein cellular protein. Data are from a single experiment that was repeated with similar results.

Figure 4. Concentration dependence of GABA transport. COS cells transfected with GAT-2 (panel A) or GAT-3 (panel B) were incubated with the indicated concentrations of [ $^3$ H]GABA for 30 seconds and the accumulated radioactivity was determined. The specific activity of the [ $^3$ H]GABA was reduced with unlabeled GABA. Data represent specific transport expressed as nmoles per minute per mg protein, and are from a single experiment that was repeated with similar results (see Text).

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Figure 5. Localization of GABA transporters. A. Northern blot analysis of mRNAs encoding GAT-2 (rB14b) and GAT-3 (rB8b). Total RNA (25  $\mu$ g) from rat brain and liver was separated by formaldehyde/agarose gel electrophoresis, blotted to nylon membranes, and hybridized at high stringency with  $^{32}$ P-labeled GABA transporter cDNAs (rB14b and rB8b, respectively). The autoradiogram was developed after a four day exposure. The locations of ribosomal RNAs are indicated at the side. The hybridizing transcripts are  $\approx$ 2.4kb (GAT-2) and  $\approx$ 4.7kb (GAT-3). B. Tissue distribution of mRNAs encoding GAT-1, GAT-2, and GAT-3 as determined by PCR. Single-stranded cDNA converted from poly A+ RNA was used for PCR amplification (30 cycles) of GABA transporter cDNA sequences. Amplified products were detected by hybridization with specific oligonucleotide probes; autoradiograms of the Southern blots are shown. GAT-1 is the neuronal GABA transporter. GAT-2 is the transporter encoded by rB8b. GAT-3 is the transporter by rB14b. Equivalent samples of poly A+ RNA (not treated with reverse transcriptase) subjected to identical PCR conditions showed no hybridization with the three probes (not shown). Cyclophilin cDNA was amplified to an equal extent from all tissues examined (not shown). Each experiment was repeated at least once with similar results.

Figure 6. Alignment of the taurine transporter with the GABA transporter GAT-1, the betaine transporter, and the glycine transporter. The twelve putative  $\alpha$ -helical membrane spanning domains (I-XII) are bracketed. Residues identical to those of the taurine transporter are shaded. Taurine is the taurine transporter encoded by rB16a; GAT-1 is the rat brain GABA transporter (21);

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Betaine is the dog betaine transporter (79); Glycine is the rat glycine transporter (68).

5       **Figure 7.** Taurine transport by COS cells transfected with clone rB16a. Non-transfected COS cells (control) or COS cells transfected with rB16a were incubated for 10 minutes (37°C) with 50nM [<sup>3</sup>H]taurine in either HBS (150mM NaCl) or in a similar solution in which Na<sup>+</sup> was replaced by equimolar Li<sup>+</sup> (Na<sup>+</sup>-free), or Cl<sup>-</sup> was replaced by acetate (Cl<sup>-</sup>-free). Data show the specific uptake of  
10       taurine, expressed as % of control cells. Each bar represents the mean±SEM of 3-7 experiments.

15       **Figure 8.** Concentration dependence of taurine transport. COS cells transfected with rB16a were incubated with the indicated concentrations of [<sup>3</sup>H]taurine for 30 seconds and the accumulated radioactivity was determined. The specific activity of [<sup>3</sup>H]taurine was reduced with  
20       unlabeled taurine. Data represent specific transport expressed as nmoles per minute per mg protein, and are from a single experiment that was repeated with similar results (see Text).

25       **Figure 9.** Localization of the taurine transporter.  
A. Tissue distribution of mRNA encoding the taurine transporter as determined by PCR. Single-stranded cDNA converted from poly A<sup>+</sup> RNA was used for PCR amplification (30 cycles) of taurine transporter cDNA from a variety of rat tissues. A plasmid containing the cloned taurine  
30       transporter was amplified under identical conditions as a control. Amplified products were detected by hybridization with an oligonucleotide probe specific to the taurine transporter; an autoradiogram of the Southern blot is shown. Equivalent samples of poly A<sup>+</sup> RNA (not  
35       treated with reverse transcriptase) subjected to

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identical PCR conditions showed no hybridization with the transporter probe (not shown), indicating that the signals obtained with cDNA were not a result of genomic DNA contamination. The experiment was repeated with similar results. B. Northern blot analysis of mRNA encoding the taurine transporter. Poly A+ RNA (5 $\mu$ g) from a variety of rat tissues was separated by formaldehyde/agarose gel electrophoresis, blotted to a nylon membrane, and hybridized at high stringency with <sup>32</sup>P-labeled taurine transporter cDNA (rB16a). The autoradiogram was developed after an overnight exposure. Size standards are indicated at the left in kilobases. The hybridizing transcript is ~6.2 kb.

Figure 10. Nucleotide Sequence and Deduced Amino Acid of Human Transporters. A. Sequence of the Human GAT-2 GABA Transporter. Nucleotides are presented in the 5' to 3' orientation and the coding region is numbered starting from the first nucleotide in a partial cDNA clone. Deduced amino acid sequence by translation of a long open reading frame is shown. B. Sequence of the Human GAT-3 GABA Transporter. Nucleotides are presented in the 5' to 3' orientation and the coding region is numbered starting from the putative initiating methionine and ending in the terminating codon. Deduced amino acid sequence by translation of a long open reading frame is shown.

Detailed Description of the Invention

5 This invention provides an isolated nucleic acid molecule encoding a mammalian GABA transporter. This invention also provides an isolated nucleic acid molecule encoding a mammalian taurine transporter. This invention further provides an isolated nucleic acid molecule encoding a human GABA transporter. As used herein, the term "isolated nucleic acid molecule" means a non-naturally occurring nucleic acid molecule that is, a molecule in a form which does not occur in nature. Examples of such an isolated nucleic acid molecule are an RNA, cDNA, or isolated genomic DNA molecule encoding a mammalian GABA, or mammalian taurine transporter. As used herein, "GABA transporter" means a molecule which, under physiologic conditions, is substantially specific for the neurotransmitter GABA, is saturable, of high affinity for GABA ( $K_m=4\mu M$ ), and exhibits pharmacological properties distinct from the neuronal GABA transporter. As used herein, "taurine transporter" means a molecule which, under physiologic conditions, is substantially specific for the neurotransmitter taurine, is saturable, and of high affinity for taurine. One embodiment of this invention is an isolated murine nucleic acid molecule encoding a GABA or taurine transporter. Such a molecule may have coding sequences substantially the same as the coding sequences shown in Figure 1A, 1B or 1C. The DNA molecules of Figures 1A (Sequence I.D. No. 1) and 1B (Seq I.D. No.3) encode the sequence of the mammalian GABA transporter genes. The DNA molecule of Figure 1C (Sequence I.D. No. 5) encodes the sequence of a mammalian taurine transporter gene. Another preferred embodiment of this invention is an isolated human nucleic acid molecule

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encoding a human GABA transporter. Such a molecule may have coding sequences substantially the same as the coding sequences shown in Figures 10A and 10B. The DNA molecules of Figures 10A (Sequence I.D. No.7) and 10B (Sequence I.D. No.9) encode the sequences of human GABA transporter genes. Another preferred embodiment of this invention is an isolated nucleic acid molecule encoding a human taurine transporter. Such a molecule may have coding sequences substantially similar to the sequence shown in Figure 1C. One means of isolating a mammalian GABA or a mammalian taurine transporter is to probe a mammalian genomic library with a natural or artificially designed DNA probe, using methods well known in the art. In the preferred embodiment of this invention, the mammalian GABA and mammalian taurine transporter are human proteins and the nucleic acid molecules encoding them are isolated from a human cDNA library or a human genomic DNA library. DNA probes derived from the rat GABA transporter genes rB14b and rB8b, and DNA probes derived from the rat taurine transporter gene rB16a are useful probes for this purpose. DNA and cDNA molecules which encode mammalian GABA or mammalian taurine transporters are used to obtain complementary genomic DNA, cDNA or RNA from human, mammalian or other animal sources, or to isolate related cDNA or genomic clones by the screening of cDNA or genomic DNA libraries, by methods described in more detail below. Transcriptional regulatory elements from the 5' untranslated region of the isolated clone, and other stability, processing, transcription, translation, and tissue specificity determining regions from the 3' and 5' untranslated regions of the isolated gene are thereby obtained.

This invention provides a method for obtaining an isolated nucleic acid molecule encoding a human taurine

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transporter which comprises using oligonucleotide primers based on the nucleic acid sequence coding for a mammalian taurine receptor and the polymerase chain reaction (PCR) to detect the presence of the nucleic acid molecule coding for the taurine transporter in a human cDNA library. PCR is carried out at reduced annealing temperatures to allow for mismatches between the nucleic acid sequences encoding the rat taurine transporter and nucleic acid sequences encoding the human taurine transporter. Amplified DNA sequences encoding a human taurine transporter are detected by hybridization at reduced hybridization stringency with radiolabelled cDNA encoding the mammalian taurine receptor. A human cDNA library identified by the above method to contain a nucleic acid molecule encoding the human taurine transporter is then screened at low hybridization stringency with the same cDNA probe encoding the mammalian taurine receptor to isolate a cDNA clone encoding a human taurine transporter. A cDNA sequence from the resulting clone can then be used to screen additionally screen a human cDNA or human genomic DNA library to obtain the entire sequence of the human homologue of the mammalian taurine transporter. Primers used in the polymerase chain reaction to initially screen human cDNA libraries to identify human cDNA libraries containing clones encoding a human taurine receptor may be composed of a plurality of degenerate primers based on the sequence of the mammalian taurine transporter. The methods of synthesizing primers, of screening cDNA libraries by PCR to identify libraries containing a cDNA clone encoding the protein of interest are well known by one of skill in the art and examples of this method for obtaining a cDNA clone encoding the human homologue of mammalian transporter are further given below. These same methods can be used to isolate cDNA and genomic DNAs

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encoding additional mammalian or human GABA transporter subtypes or taurine transporter subtypes encoded by different genes or encoded by the same gene and generated by alternative splicing of the RNA or rearrangement of the genomic DNA.

This invention provides an isolated nucleic acid molecule which has been so mutated as to be incapable of encoding a molecule having normal transporter activity, and not expressing native transporter. An example of a mutated nucleic acid molecule provided by this invention is an isolated nucleic acid molecule which has an in-frame stop codon inserted into the coding sequence such that the transcribed RNA is not translated into a protein having normal transporter activity.

This invention further provides a cDNA molecule encoding a mammalian GABA transporter, wherein the cDNA molecule has a coding sequence substantially the same as the coding sequence shown in Figure 1A or 1B. (Sequence I.D. Nos. 1 or 3). This invention also provides a cDNA molecule encoding a mammalian taurine transporter, wherein the cDNA molecule has a coding sequence substantially the same as the coding sequence shown in Figure 1C. (Sequence I.D. No. 5). This invention also provides a cDNA molecule encoding a human GABA transporter, wherein the cDNA molecule has a coding sequence substantially the same as the coding sequence shown in Figures 10A (Sequence I.D. No. 7) and 10B (Sequence I.D. No. 9). These molecules and their equivalents were obtained by the means described above.

This invention also provides an isolated protein which is a mammalian GABA transporter. This invention further provides an isolated protein which is a mammalian taurine



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transporter. In one embodiment of this invention, the protein is a murine GABA transporter protein having an amino acid sequence substantially similar to the amino acid sequence shown in Figures 1A (Seq. I.D. Nos. 1 and 2) or 1B (Seq. I.D. Nos. 3 and 4). In another embodiment of this invention, the protein is a murine taurine transporter protein having an amino acid sequence substantially similar to the amino acid sequence shown in Figure 1C (Seq. I.D. Nos. 5 and 6). In a preferred embodiment of this invention, the protein is a human GABA transporter protein having an amino acid sequence substantially the same as the sequence shown in Figure 10A (Sequence I.D. Nos. 7 and 8) and Figure 10B (Sequence I.D. Nos. 9 and 10). Another preferred embodiment of this invention, the protein is a human taurine transporter protein having an amino acid sequence substantially similar to the amino acid sequence shown in Figure 1C (Seq. I.D. Nos. 5 and 6). As used herein, the term "isolated protein" is intended to encompass a protein molecule free of other cellular components. One means for obtaining an isolated GABA or taurine transporter is to express DNA encoding the transporter in a suitable host, such as a bacterial, yeast, or mammalian cell, using methods well known to those skilled in the art, and recovering the transporter protein after it has been expressed in such a host, again using methods well known in the art. The transporter may also be isolated from cells which express it, in particular from cells which have been transfected with the expression vectors described below in more detail.

This invention also provides a vector comprising an isolated nucleic acid molecule such as DNA, RNA, or cDNA, encoding a mammalian GABA transporter. This invention also provides a vector comprising an isolated nucleic

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acid molecule such as DNA, RNA, or cDNA, encoding a mammalian taurine transporter. This invention also provides a vector comprising an isolated nucleic acid molecule such as DNA, RNA, or cDNA, encoding a human GABA transporter. This invention also provides a vector comprising an isolated nucleic acid molecule such as DNA, RNA, or cDNA, encoding a human taurine transporter. Examples of vectors are viruses such as bacteriophages (such as phage lambda), cosmids, plasmids (such as pUC18, available from Pharmacia, Piscataway, NJ), and other recombination vectors. Nucleic acid molecules are inserted into vector genomes by methods well known to those skilled in the art. Examples of such plasmids are plasmids comprising cDNA having a coding sequence substantially the same as: the coding sequence shown in Figure 1A (Seq. I.D. No. 1) and designated clone pEVJB-rB14b deposited under ATCC Accession No. 75203, the coding sequence shown in Figure 1B (Seq. I.D. No. 3) and designated clone pEVJB-rB8b deposited under ATCC Accession No. 75201, the coding sequence shown in Figure 1C (Seq. I.D. No. 5) and designated pEVJB-rB16a deposited under ATCC Accession No. 75202, the coding sequence shown in Figure 10A, (Sequence I.D. No. 7) designated pBluescript-hHE7a and pBluescript-hS3a and deposited under ATCC Accession Nos.            and            , respectively, or the coding sequence shown in Figure 10B (SEQ. I.D. No. 9) and designated pcEXV-hGAT-3 and deposited under ATCC Accession No.            . Alternatively, to obtain these vectors, insert and vector DNA can both be exposed to a restriction enzyme to create complementary ends on both molecules which base pair with each other and are then ligated together with a ligase. Alternatively, linkers can be ligated to the insert DNA which correspond to a restriction site in the vector DNA, which is then

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digested with the restriction enzyme which cuts at that site. Other means are also available.

5 This invention also provides vectors comprising a DNA molecule encoding a mammalian GABA transporter and vectors comprising a DNA molecule encoding a mammalian taurine transporter, adapted for expression in a bacterial cell, a yeast cell, or a mammalian cell which additionally comprise the regulatory elements necessary  
10 for expression of the DNA in the bacterial, yeast, or mammalian cells so located relative to the DNA encoding a mammalian GABA transporter or to the DNA encoding a mammalian taurine transporter as to permit expression thereof. DNA having coding sequences substantially the same as the coding sequence shown in Figure 1A or Figure 1B may usefully be inserted into the vectors to express mammalian GABA transporters. DNA having coding sequences substantially the same as the coding sequence shown in Figure 1C may usefully be inserted into the vectors to express mammalian taurine transporters. This invention also provides vectors comprising a DNA molecule encoding a human GABA transporter adapted for expression in a bacterial cell, a yeast cell, or a mammalian cell which additionally comprise the regulatory elements necessary  
25 for expression of the DNA in the bacterial, yeast, or mammalian cells so located relative to the DNA encoding a human GABA transporter as to permit expression thereof. DNA having coding sequences substantially the same as the coding sequence shown in Figures 10A and 10B may usefully be inserted into the vectors to express human GABA transporters. This invention also provides vectors comprising a DNA molecule encoding a human taurine transporter adapted for expression in a bacterial cell, a yeast cell, or a mammalian cell which additionally  
35 comprise the regulatory elements necessary for expression

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of the DNA in the bacterial, yeast, or mammalian cells so located relative to the DNA encoding a human taurine transporter as to permit expression thereof. Regulatory elements required for expression include promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector includes a promoter such as the lac promoter and for transcription initiation the Shine-Dalgarno sequence and the start codon AUG (Maniatis, et al., Molecular Cloning, Cold Spring Harbor Laboratory, 1982). Similarly, a eukaryotic expression vector includes a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Such vectors may be obtained commercially or assembled from the sequences described by methods well known in the art, for example the methods described above for constructing vectors in general. Expression vectors are useful to produce cells that express the transporter. Certain uses for such cells are described in more detail below.

In one embodiment of this invention a plasmid is adapted for expression in a bacterial, yeast, or, in particular, a mammalian cell wherein the plasmid comprises a DNA molecule encoding a mammalian GABA transporter or a DNA molecule encoding a mammalian taurine transporter and the regulatory elements necessary for expression of the DNA in the bacterial, yeast, or mammalian cell so located relative to the DNA encoding a mammalian GABA transporter or to the DNA encoding a mammalian taurine transporter as to permit expression thereof. In another embodiment of this invention a plasmid is adapted for expression in a bacterial, yeast, or, in particular, a mammalian cell wherein the plasmid comprises a DNA molecule encoding a

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human GABA transporter or human taurine transporter and the regulatory elements necessary for expression of the DNA in the bacterial, yeast, or mammalian cell so located relative to the DNA encoding a human GABA transporter or human taurine transporter as to permit expression thereof. Suitable plasmids may include, but are not limited to plasmids adapted for expression in a mammalian cell, e.g., EVJB or EXV. Examples of such plasmids adapted for expression in a mammalian cell are plasmids comprising cDNA having coding sequences substantially the same as the coding sequence shown in Figures 1A, 1B, 1C, 10A and 10B and the regulatory elements necessary for expression of the DNA in the mammalian cell. These plasmids have been designated pEVJB-rB14b deposited under ATCC Accession No.75203, pEVJB-rB8b deposited under ATCC Accession No.75201, pEVJB-rB16a deposited under ATCC Accession No.75202, pBluescript-hHE7a and pBluescript-hS3a deposited under ATCC Accession Nos.        and        , and pcEXV-hGAT-3 deposited under ATCC accession No.        , respectively. Those skilled in the art will readily appreciate that numerous plasmids adapted for expression in a mammalian cell which comprise DNA encoding a mammalian GABA transporter, a mammalian taurine transporter, a human GABA transporter or human taurine transporter and the regulatory elements necessary to express such DNA in the mammalian cell may be constructed utilizing existing plasmids and adapted as appropriate to contain the regulatory elements necessary to express the DNA in the mammalian cell. The plasmids may be constructed by the methods described above for expression vectors and vectors in general, and by other methods well known in the art.

The deposits discussed supra were made pursuant to, and in satisfaction of, the provisions of the Budapest Treaty

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on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852.

5 This invention provides a mammalian cell comprising a DNA molecule encoding a mammalian GABA transporter or a DNA molecule encoding a mammalian taurine transporter, such as a mammalian cell comprising a plasmid adapted for  
10 expression in a mammalian cell, which comprises a DNA molecule encoding a mammalian GABA transporter or a DNA encoding a mammalian taurine transporter and the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the DNA  
15 encoding a mammalian transporter as to permit expression thereof. This invention also provides a mammalian cell comprising a DNA molecule encoding a human GABA transporter or a human taurine transporter, such as a mammalian cell comprising a plasmid adapted for  
20 expression in a mammalian cell, which comprises a DNA molecule encoding a human GABA transporter or DNA encoding a human taurine transporter and the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the DNA encoding a  
25 human transporter as to permit expression thereof. Numerous mammalian cells may be used as hosts, including, but not limited to, the mouse fibroblast cell NIH3T3, CHO cells, HeLa cells, Ltk<sup>-</sup> cells, Cos cells, etc. Expression plasmids such as that described supra may be  
30 used to transfect mammalian cells by methods well known in the art such as calcium phosphate precipitation, or DNA encoding these transporters may be otherwise introduced into mammalian cells, e.g., by microinjection, to obtain mammalian cells which comprise DNA, e.g., cDNA  
35 or a plasmid, encoding a mammalian GABA transporter,

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encoding a mammalian taurine transporter or encoding a human GABA transporter.

5 This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a mammalian GABA transporter, for example with a coding sequence included within the sequences shown in 10 Figures 1A and 1B. This invention also provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a taurine 15 transporter, for example with a coding sequence included within the sequence shown in Figure 1C. This invention also provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human 20 GABA transporter, for example with a coding sequence included within the sequence shown in Figures 10A and 10B. This invention also provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a 25 sequence included within the sequence of a nucleic acid molecule encoding a human taurine transporter, for example with a coding sequence substantially similar to the coding sequence included within the sequence shown in 30 Figure 1C. As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs. Nucleic acid 35 probe technology is well known to those skilled in the

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art who will readily appreciate that such probes may vary greatly in length and may be labeled with a detectable label, such as a radioisotope or fluorescent dye, to facilitate detection of the probe. Detection of nucleic acid encoding a mammalian GABA transporter, mammalian taurine transporter, human GABA transporter or human taurine transporter is useful as a diagnostic test for any disease process in which levels of expression of the corresponding GABA or taurine transporter are altered.

5 DNA probe molecules are produced by insertion of a DNA molecule which encodes the mammalian GABA transporter, the mammalian taurine transporter, the human GABA transporter or the human taurine transporter or fragments thereof into suitable vectors, such as plasmids or bacteriophages, followed by insertion into suitable bacterial host cells and replication and harvesting of the DNA probes, all using methods well known in the art.

10 For example, the DNA may be extracted from a cell lysate using phenol and ethanol, digested with restriction enzymes corresponding to the insertion sites of the DNA into the vector (discussed above), electrophoresed, and cut out of the resulting gel. Examples of such DNA molecules are shown in Figures 1A, 1B, 1C, 10A and 10B.

15 The probes are useful for 'in situ' hybridization or in order to locate tissues which express this gene family, or for other hybridization assays for the presence of these genes or their mRNA in various biological tissues. In addition, synthesized oligonucleotides (produced by a DNA synthesizer) complementary to the sequence of a DNA

20 molecule which encodes a mammalian GABA transporter or a mammalian taurine transporter or complementary to the sequence of a DNA molecule which encodes a human GABA transporter or human taurine transporter, are useful as probes for these genes, for their associated mRNA, or for

25 the isolation of related genes by homology screening of

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genomic or cDNA libraries, or by the use of amplification techniques such as the Polymerase Chain Reaction.

5 This invention also provides a method of detecting expression of a GABA transporter on the surface of a cell by detecting the presence of mRNA coding for a GABA transporter. This invention also provides a method of detecting expression of a taurine transporter on the surface of the cell by detecting the presence of mRNA  
10 coding for a taurine transporter. This invention further provides a method of detecting the expression of a human GABA or human taurine transporter on the surface of the cell by detecting the presence of mRNA coding for the corresponding GABA or taurine transporter. These methods  
15 comprise obtaining total mRNA from the cell using methods well known in the art and contacting the mRNA so obtained with a nucleic acid probe as described hereinabove, under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the  
20 expression of the transporter by the cell. Hybridization of probes to target nucleic acid molecules such as mRNA molecules employs techniques well known in the art. However, in one embodiment of this invention, nucleic acids are extracted by precipitation from lysed cells and  
25 the mRNA is isolated from the extract using a column which binds the poly-A tails of the mRNA molecules (48). The mRNA is then exposed to radioactively labelled probe on a nitrocellulose membrane, and the probe hybridizes to and thereby labels complementary mRNA sequences. Binding  
30 may be detected by autoradiography or scintillation counting. However, other methods for performing these steps are well known to those skilled in the art, and the discussion above is merely an example.

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This invention provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a mammalian GABA transporter so as to prevent translation of the mammalian GABA transporter. This invention also provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a mammalian taurine transporter so as to prevent translation of the mammalian taurine transporter. This invention provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a human GABA transporter so as to prevent translation of the human GABA transporter. This invention also provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a human taurine transporter so as to prevent translation of the human taurine transporter. As used herein, the phrase "binding specifically" means the ability of an antisense oligonucleotide to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs. The antisense oligonucleotide may have a sequence capable of binding specifically with any sequences of the cDNA molecules whose sequences are shown in Figures 1A, 1B, 1C, 10A and 10B. A particular example of an antisense oligonucleotide is an antisense oligonucleotide comprising chemical analogues of nucleotides.

This invention also provides a pharmaceutical composition comprising an effective amount of the oligonucleotide described above effective to reduce expression of a mammalian GABA transporter by passing through a cell

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membrane and binding specifically with mRNA encoding a mammalian GABA transporter in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane. This invention provides a pharmaceutical composition comprising an effective amount of the oligonucleotide described above effective to reduce expression of a mammalian taurine transporter by passing through a cell membrane and binding specifically with mRNA encoding a mammalian taurine transporter in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane. This invention also provides a pharmaceutical composition comprising an effective amount of the oligonucleotide described above effective to reduce expression of a human GABA transporter by passing through a cell membrane and binding specifically with mRNA encoding a human GABA transporter in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane. This invention also provides a pharmaceutical composition comprising an effective amount of the oligonucleotide described above effective to reduce expression of a human taurine transporter by passing through a cell membrane and binding specifically with mRNA encoding a human taurine transporter in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane. As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. The oligonucleotide may be coupled to a substance which inactivates mRNA, such as a

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ribozyme. The pharmaceutically acceptable hydrophobic carrier capable of passing through cell membranes may also comprise a structure which binds to a transporter specific for a selected cell type and is thereby taken up  
5 by cells of the selected cell type. The structure may be part of a protein known to bind a cell-type specific transporter, for example an insulin molecule, which would target pancreatic cells. DNA molecules having coding sequences substantially the same as the coding sequence  
10 shown in Figures 1A, 1B, 1C, 10A or 10B may be used as the oligonucleotides of the pharmaceutical composition.

This invention also provides a method of treating abnormalities which are alleviated by reduction of  
15 expression of a GABA transporter. This method comprises administering to a subject an effective amount of the pharmaceutical composition described above effective to reduce expression of the GABA transporter by the subject. This invention further provides a method of treating an  
20 abnormal condition related to GABA transporter activity which comprises administering to a subject an amount of the pharmaceutical composition described above effective to reduce expression of the GABA transporter by the subject. Examples of such abnormal conditions are  
25 epilepsy and generalized anxiety. This invention also provides a method of treating abnormalities which are alleviated by reduction of expression of a taurine transporter. This method comprises administering to a subject an effective amount of the pharmaceutical  
30 composition described above effective to reduce expression of the taurine transporter by the subject. This invention further provides a method of treating an abnormal condition related to taurine transporter activity which comprises administering to a subject an  
35 amount of the pharmaceutical composition described above

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effective to reduce expression of the taurine transporter by the subject. Examples of such abnormal conditions are epilepsy, migraine, and ischemia.

5 Antisense oligonucleotide drugs inhibit translation of mRNA encoding these transporters. Synthetic antisense oligonucleotides, or other antisense chemical structures are designed to bind to mRNA encoding a GABA transporter or to mRNA encoding a taurine transporter and inhibit  
10 translation of mRNA and are useful as drugs to inhibit expression of GABA transporter genes or taurine transporter genes in patients. This invention provides a means to therapeutically alter levels of expression of mammalian GABA or taurine transporters by the use of a  
15 synthetic antisense oligonucleotide drug (SAOD) which inhibits translation of mRNA encoding these transporters. Synthetic antisense oligonucleotides, or other antisense chemical structures designed to recognize and selectively bind to mRNA, are constructed to be complementary to  
20 portions of the nucleotide sequences shown in Figures 1A, 1B, 1C, 10A or 10B of DNA, RNA or of chemically modified, artificial nucleic acids. The SAOD is designed to be stable in the blood stream for administration to patients by injection, or in laboratory cell culture conditions, for administration to cells removed from the  
25 patient. The SAOD is designed to be capable of passing through cell membranes in order to enter the cytoplasm of the cell by virtue of physical and chemical properties of the SAOD which render it capable of passing through cell  
30 membranes (e.g., by designing small, hydrophobic SAOD chemical structures) or by virtue of specific transport systems in the cell which recognize and transport the SAOD into the cell. In addition, the SAOD can be designed for administration only to certain selected cell  
35 populations by targeting the SAOD to be recognized by

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specific cellular uptake mechanisms which bind and take up the SAOD only within certain selected cell populations. For example, the SAOD may be designed to bind to a transporter found only in a certain cell type, as discussed above. The SAOD is also designed to recognize and selectively bind to the target mRNA sequence, which may correspond to a sequence contained within the sequences shown in Figures 1A, 1B, 1C, 10A or 10B by virtue of complementary base pairing to the mRNA. Finally, the SAOD is designed to inactivate the target mRNA sequence by any of three mechanisms: 1) by binding to the target mRNA and thus inducing degradation of the mRNA by intrinsic cellular mechanisms such as RNase I digestion, 2) by inhibiting translation of the mRNA target by interfering with the binding of translation-regulating factors or of ribosomes, or 3) by inclusion of other chemical structures, such as ribozyme sequences or reactive chemical groups, which either degrade or chemically modify the target mRNA. Synthetic antisense oligonucleotide drugs have been shown to be capable of the properties described above when directed against mRNA targets (11,76). In addition, coupling of ribozymes to antisense oligonucleotides is a promising strategy for inactivating target mRNA (60). An SAOD serves as an effective therapeutic agent if it is designed to be administered to a patient by injection, or if the patient's target cells are removed, treated with the SAOD in the laboratory, and replaced in the patient. In this manner, an SAOD serves as a therapy to reduce transporter expression in particular target cells of a patient, in any clinical condition which may benefit from reduced expression of GABA or taurine transporters.

This invention provides an antibody directed to the mammalian GABA transporter. This antibody may comprise,

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for example, a monoclonal antibody directed to an epitope of a mammalian GABA transporter present on the surface of a cell, the epitope having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the mammalian GABA transporter included in the amino acid sequence shown in Figures 1A or 1B. This invention provides an antibody directed to the mammalian taurine transporter. This antibody may comprise, for example, a monoclonal antibody directed to an epitope of a mammalian taurine transporter present on the surface of a cell, the epitope having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the mammalian taurine transporter included in the amino acid sequence shown in Figure 1C. This invention provides an antibody directed to a human GABA transporter. This antibody may comprise, for example, a monoclonal antibody directed to an epitope of a human GABA transporter present on the surface of a cell, the epitope having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the human GABA transporter included in the amino acid sequence shown in Figures 10A and 10B. This invention provides an antibody directed to a human taurine transporter. This antibody may comprise, for example, a monoclonal antibody directed to an epitope of a human taurine transporter present on the surface of a cell, the epitope having an amino acid sequence substantially similar to the amino acid sequence for a cell surface epitope of the mammalian taurine transporter shown in Figure 1C. Amino acid sequences may be analyzed by methods well known to those skilled in the art to determine whether they produce hydrophobic or hydrophilic regions in the proteins which they build. In the case of cell membrane proteins, hydrophobic regions are well known to form the part of the protein that is inserted

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into the lipid bilayer which forms the cell membrane, while hydrophilic regions are located on the cell surface, in an aqueous environment. Therefore antibodies to the hydrophilic amino acid sequences shown in Figures 1A or 1B will bind to a surface epitope of a mammalian GABA transporter, and antibodies to the hydrophilic amino acid sequences shown in Figure 1C will bind to a surface epitope of a mammalian taurine transporter, as described. Antibodies to the hydrophilic amino acid sequences shown in Figures 10A or 10B will bind to a surface epitope of a human GABA transporter. Antibodies directed to conserved hydrophilic amino acid sequences specific to a mammalian taurine transporter will bind to a surface epitope of a human taurine transporter. Antibodies directed to mammalian or human transporters may be serum-derived or monoclonal and are prepared using methods well known in the art. For example, monoclonal antibodies are prepared using hybridoma technology by fusing antibody producing B cells from immunized animals with myeloma cells and selecting the resulting hybridoma cell line producing the desired antibody. Cells such as NIH3T3 cells or Ltk<sup>-</sup> cells may be used as immunogens to raise such an antibody. Alternatively, synthetic peptides may be prepared using commercially available machines and the amino acid sequences shown in Figures 1A, 1B, 1C, 10A and 10B. As a still further alternative, DNA, such as a cDNA or a fragment thereof, may be cloned and expressed and the resulting polypeptide recovered and used as an immunogen. These antibodies are useful to detect the presence of mammalian transporters encoded by the isolated DNA, or to inhibit the function of the transporters in living animals, in humans, or in biological tissues or fluids isolated from animals or humans.

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This invention also provides a pharmaceutical composition which comprises an effective amount of an antibody directed to an epitope of the mammalian transporter, effective to block binding of naturally occurring substrates to the transporter, and a pharmaceutically acceptable carrier. A monoclonal antibody directed to an epitope of a mammalian GABA transporter present on the surface of a cell which has an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the mammalian GABA transporter included in the amino acid sequences shown in Figures 1A and 1B is useful for this purpose. A monoclonal antibody directed to an epitope of a mammalian taurine transporter present on the surface of a cell which has an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the mammalian taurine transporter included in the amino acid sequence shown in Figure 1C is also useful for this purpose.

This invention also provides a pharmaceutical composition which comprises an effective amount of an antibody directed to an epitope of the human transporter, effective to block binding of naturally occurring substrates to the transporter, and a pharmaceutically acceptable carrier. A monoclonal antibody directed to an epitope of a human GABA transporter present on the surface of a cell which has an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the human GABA transporter included in the amino acid sequences shown in Figures 10A or 10B is useful for this purpose.

This invention also provides a pharmaceutical composition which comprises an effective amount of an antibody directed to an epitope of a human taurine transporter,

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effective to block binding of naturally occurring substrates to the human taurine transporter, and a pharmaceutically acceptable carrier. A monoclonal antibody directed to a conserved epitope specific to a mammalian taurine transporter present on the surface of a cell which has an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the mammalian taurine transporter included in the amino acid sequence shown in Figure 1C is useful for this purpose.

This invention also provides a method of treating abnormalities in a subject which are alleviated by reduction of expression of a mammalian transporter which comprises administering to the subject an effective amount of the pharmaceutical composition described above effective to block binding of naturally occurring substrates to the transporter and thereby alleviate abnormalities resulting from overexpression of a mammalian transporter. Binding of the antibody to the transporter prevents the transporter from functioning, thereby neutralizing the effects of overexpression. The monoclonal antibodies described above are both useful for this purpose. This invention additionally provides a method of treating an abnormal condition related to an excess of transporter activity which comprises administering to a subject an amount of the pharmaceutical composition described above effective to block binding of naturally occurring substrates to the transporter and thereby alleviate the abnormal condition. Some examples of abnormal conditions associated with excess GABA transporter activity are epilepsy and generalized anxiety. Excess taurine transporter activity associated disorders are epilepsy, migraine, and ischemia.

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This invention provides methods of detecting the presence of a GABA or a taurine transporter on the surface of a cell which comprises contacting the cell with an antibody directed to the mammalian GABA transporter or an antibody directed to the mammalian taurine transporter, under conditions permitting binding of the antibody to the transporter, detecting the presence of the antibody bound to the cell, and thereby the presence of the mammalian GABA transporter or the presence of the taurine transporter on the surface of the cell. Such methods are useful for determining whether a given cell is defective in expression of GABA transporters or is defective in expression of taurine transporters on the surface of the cell. Bound antibodies are detected by methods well known in the art, for example by binding fluorescent markers to the antibodies and examining the cell sample under a fluorescence microscope to detect fluorescence on a cell indicative of antibody binding. The monoclonal antibodies described above are useful for this purpose.

This invention provides a transgenic nonhuman mammal expressing DNA encoding a mammalian GABA transporter and a transgenic nonhuman mammal expressing DNA encoding a mammalian taurine transporter. This invention further provides a transgenic nonhuman mammal expressing DNA encoding a human GABA transporter and a transgenic nonhuman mammal expressing DNA encoding a human taurine transporter. This invention also provides a transgenic nonhuman mammal expressing DNA encoding a mammalian GABA transporter so mutated as to be incapable of normal transporter activity, and not expressing native GABA transporter and a transgenic nonhuman mammal expressing DNA encoding a mammalian taurine transporter so mutated as to be incapable of normal transporter activity, and not expressing native taurine transporter. This invention

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further provides a transgenic nonhuman mammal expressing DNA encoding a human GABA transporter so mutated as to be incapable of normal transporter activity, and not expressing native GABA transporter and a transgenic nonhuman mammal expressing DNA encoding a human taurine transporter so mutated as to be incapable of normal transporter activity, and not expressing native taurine transporter.

10 This invention provides a transgenic nonhuman mammal whose genome comprises DNA encoding a mammalian GABA transporter so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a GABA transporter and which hybridizes to mRNA encoding a GABA transporter thereby reducing its translation and a transgenic nonhuman mammal whose genome comprises DNA encoding a mammalian taurine transporter so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a taurine transporter and which hybridizes to mRNA encoding a taurine transporter thereby reducing its translation. This invention further provides a transgenic nonhuman mammal whose genome comprises DNA encoding a human GABA transporter so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a GABA transporter and which hybridizes to mRNA encoding a GABA transporter thereby reducing its translation and a transgenic nonhuman mammal whose genome comprises DNA encoding a human taurine transporter so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a taurine transporter and which hybridizes to mRNA encoding a taurine transporter thereby reducing its translation. The DNA may additionally comprise an inducible promoter or additionally comprise tissue specific regulatory elements, so that expression can be

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induced, or restricted to specific cell types. Examples of DNA are DNA or cDNA molecules having a coding sequence substantially the same as the coding sequences shown in Figures 1A, 1B, 1C, 10A and 10B. An example of a transgenic animal is a transgenic mouse. Examples of tissue specificity-determining regions are the metallothionein promoter (46,83) and the L7 promoter (84).

Animal model systems which elucidate the physiological and behavioral roles of mammalian transporters are produced by creating transgenic animals in which the expression of a transporter is either increased or decreased, or the amino acid sequence of the expressed transporter protein is altered, by a variety of techniques. Examples of these techniques include, but are not limited to: 1) Insertion of normal or mutant versions of DNA encoding a mammalian transporter or homologous animal versions of these genes, by microinjection, retroviral infection or other means well known to those skilled in the art, into appropriate fertilized embryos in order to produce a transgenic animal (24) or 2) Homologous recombination (7,82) of mutant or normal, human or animal versions of these genes with the native gene locus in transgenic animals to alter the regulation of expression or the structure of these transporters. The technique of homologous recombination is well known in the art. It replaces the native gene with the inserted gene and so is useful for producing an animal that cannot express native transporter but does express, for example, an inserted mutant transporter, which has replaced the native transporter in the animal's genome by recombination, resulting in underexpression of the transporter. Microinjection adds genes to the genome, but does not remove them, and so is useful for

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producing an animal which expresses its own and added transporters, resulting in overexpression of the transporter.

5 One means available for producing a transgenic animal, with a mouse as an example, is as follows: Female mice are mated, and the resulting fertilized eggs are dissected out of their oviducts. The eggs are stored in an appropriate medium such as M2 medium (24). DNA or  
10 cDNA encoding a mammalian transporter is purified from a vector (such as plasmids EVJB-rB14b, EVJB-rB8b, or EVJB-rB16a described above) by methods well known in the art. Inducible promoters may be fused with the coding region of the DNA to provide an experimental means to regulate  
15 expression of the trans-gene. Alternatively or in addition, tissue specific regulatory elements may be fused with the coding region to permit tissue-specific expression of the trans-gene. The DNA, in an appropriately buffered solution, is put into a  
20 microinjection needle (which may be made from capillary tubing using a pipet puller) and the egg to be injected is put in a depression slide. The needle is inserted into the pronucleus of the egg, and the DNA solution is injected. The injected egg is then transferred into the  
25 oviduct of a pseudopregnant mouse (a mouse stimulated by the appropriate hormones to maintain pregnancy but which is not actually pregnant), where it proceeds to the uterus, implants, and develops to term. As noted above, microinjection is not the only method for inserting DNA  
30 into the egg cell, and is used here only for exemplary purposes.

Since the normal action of transporter-specific drugs is to activate or to inhibit the transporter, the transgenic  
35 animal model systems described above are useful for

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testing the biological activity of drugs directed against these transporters even before such drugs become available. These animal model systems are useful for predicting or evaluating possible therapeutic applications of drugs which activate or inhibit these transporters by inducing or inhibiting expression of the native or trans-gene and thus increasing or decreasing expression of normal or mutant transporters in the living animal. Thus, a model system is produced in which the biological activity of drugs directed against these transporters are evaluated before such drugs become available. The transgenic animals which over or under produce the transporter indicate by their physiological state whether over or under production of the transporter is therapeutically useful. It is therefore useful to evaluate drug action based on the transgenic model system. One use is based on the fact that it is well known in the art that a drug such as an antidepressant acts by blocking neurotransmitter uptake, and thereby increases the amount of neurotransmitter in the synaptic cleft. The physiological result of this action is to stimulate the production of less transporter by the affected cells, leading eventually to underexpression. Therefore, an animal which underexpresses transporter is useful as a test system to investigate whether the actions of such drugs which result in under expression are in fact therapeutic. Another use is that if overexpression is found to lead to abnormalities, then a drug which down-regulates or acts as an antagonist to the transporter is indicated as worth developing, and if a promising therapeutic application is uncovered by these animal model systems, activation or inhibition of the GABA transporter is achieved therapeutically either by producing agonist or antagonist drugs directed against

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these GABA transporters or by any method which increases or decreases the expression of these transporters in man.

5 Further provided by this invention is a method of determining the physiological effects of expressing varying levels of mammalian transporters which comprises producing a transgenic nonhuman animal whose levels of mammalian transporter expression are varied by use of an inducible promoter which regulates mammalian transporter  
10 expression. This invention also provides a method of determining the physiological effects of expressing varying levels of mammalian transporters which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of mammalian transporter.  
15 Such animals may be produced by introducing different amounts of DNA encoding a mammalian transporter into the oocytes from which the transgenic animals are developed.

20 This invention provides a method of determining the physiological effects of expressing varying levels of human transporters which comprises producing a transgenic nonhuman animal whose levels of human transporter expression are varied by use of an inducible promoter which regulates transporter expression. This invention  
25 also provides a method of determining the physiological effects of expressing varying levels of human transporters which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of the human transporter. Such animals may be  
30 produced by introducing different amounts of DNA encoding a human transporter into the oocytes from which the transgenic animals are developed.

35 This invention also provides a method for identifying a substance capable of alleviating abnormalities resulting



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from overexpression of a mammalian transporter comprising administering the substance to a transgenic nonhuman mammal expressing at least one artificially introduced DNA molecule encoding a mammalian transporter and  
5 determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overexpression of a mammalian transporter. This invention also provides a method for identifying a substance capable of alleviating  
10 abnormalities resulting from overexpression of a human transporter comprising administering the substance to a transgenic nonhuman mammal expressing at least one artificially introduced DNA molecule encoding a human transporter and determining whether the substance  
15 alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overexpression of a human transporter. As used herein, the term "substance" means a compound or composition which may be natural, synthetic, or a product  
20 derived from screening. Examples of DNA molecules are DNA or cDNA molecules having a coding sequence substantially the same as the coding sequences shown in Figures 1A, 1B, 1C, 10A or 10B.

25 This invention provides a pharmaceutical composition comprising an amount of the substance described supra effective to alleviate the abnormalities resulting from overexpression of GABA transporter and a pharmaceutically acceptable carrier. This invention also provides a  
30 pharmaceutical composition comprising an amount of the substance described supra effective to alleviate the abnormalities resulting from overexpression of taurine transporter and a pharmaceutically acceptable carrier. This invention further provides a pharmaceutical  
35 composition comprising an amount of the substance

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described supra effective to alleviate the abnormalities resulting from overexpression of a human GABA or human taurine transporter and a pharmaceutically acceptable carrier.

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This invention also provides a method for treating the abnormalities resulting from overexpression of a mammalian transporter which comprises administering to a subject an amount of the pharmaceutical composition described above effective to alleviate the abnormalities resulting from overexpression of a mammalian transporter. This invention further provides a method for treating the abnormalities resulting from overexpression of a human GABA or human taurine transporter which comprises administering to a subject an amount of the pharmaceutical composition described above effective to alleviate the abnormalities resulting from overexpression of a human GABA or taurine transporter.

20 This invention provides a method for identifying a substance capable of alleviating the abnormalities resulting from underexpression of a mammalian transporter comprising administering the substance to the transgenic nonhuman mammal described above which expresses only nonfunctional mammalian transporter and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of underexpression of a mammalian transporter. This invention further provides a method for identifying a substance capable of alleviating the abnormalities resulting from underexpression of a human GABA or human taurine transporter comprising administering the substance to the transgenic nonhuman mammal described above which expresses only nonfunctional human GABA or human taurine transporter and determining

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whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of underexpression of a human GABA or human taurine transporter.

5

This invention also provides a pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of transporter and a pharmaceutically acceptable carrier.

10

This invention also provides a pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of a human GABA or human taurine transporter and a pharmaceutically acceptable carrier.

15

This invention provides a method for treating the abnormalities resulting from underexpression of a mammalian transporter which comprises administering to a subject an amount of the pharmaceutical composition described above effective to alleviate the abnormalities resulting from underexpression of a mammalian transporter. This invention further provides a method for treating the abnormalities resulting from underexpression of a human GABA or human taurine transporter which comprises administering to a subject an amount of the pharmaceutical composition described above effective to alleviate the abnormalities resulting from underexpression of a human GABA or human taurine transporter.

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This invention provides a method for diagnosing a predisposition to a disorder associated with the expression of a specific mammalian transporter allele which comprises: a) obtaining DNA of subjects suffering from the disorder; b) performing a restriction digest of

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the DNA with a panel of restriction enzymes; c) electrophoretically separating the resulting DNA fragments on a sizing gel; d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a mammalian transporter and labelled with a detectable marker; e) detecting labelled bands which have hybridized to the DNA encoding a mammalian transporter labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder; f) preparing DNA obtained for diagnosis by steps a-e; and g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and thereby to diagnose predisposition to the disorder if the patterns are the same. This method may also be used to diagnose a disorder associated with the expression of a specific mammalian transporter allele.

This invention provides a method for diagnosing a predisposition to a disorder associated with the expression of a specific human GABA or human taurine transporter allele which comprises: a) obtaining DNA of subjects suffering from the disorder; b) performing a restriction digest of the DNA with a panel of restriction enzymes; c) electrophoretically separating the resulting DNA fragments on a sizing gel; d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a human GABA or human taurine transporter and labelled with a detectable marker; e) detecting labelled bands which have hybridized to the DNA encoding a human GABA or human taurine transporter labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects

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suffering from the disorder; f) preparing DNA obtained for diagnosis by steps a-e; and g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and thereby to diagnose predisposition to the disorder if the patterns are the same. This method may also be used to diagnose a disorder associated with the expression of a specific human GABA or human taurine transporter allele.

This invention provides a method of preparing the isolated transporter which comprises inducing cells to express transporter, recovering the transporter from the resulting cells, and purifying the transporter so recovered. An example of an isolated GABA transporter is an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figures 1A or 1B. An example of an isolated taurine transporter is an isolated protein having substantially the same amino acid sequence shown in Figure 1C. This invention further provides a method for preparing an isolated human GABA transporter which comprises inducing cells to express the human GABA transporter, recovering the human GABA transporter from the resulting cells, and purifying the human GABA transporter so recovered. An example of an isolated human GABA transporter is an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figures 10A or 10B. This invention further provides a method for preparing an isolated human taurine transporter which comprises inducing cells to express the human taurine transporter, recovering the human taurine transporter from the resulting cells, and purifying the human taurine transporter so recovered. An example of an isolated

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human taurine transporter is an isolated protein having an amino acid sequence substantially similar to the amino acid sequence of a mammalian taurine transporter shown in Figure 1C. For example, cells can be induced to

5 express transporters by exposure to substances such as hormones. The cells can then be homogenized and the transporter isolated from the homogenate using an affinity column comprising, for example, GABA, taurine, or another substance which is known to bind to the

10 transporter. The resulting fractions can then be purified by contacting them with an ion exchange column, and determining which fraction contains transporter activity or binds anti-transporter antibodies.

15 This invention provides a method of preparing the isolated mammalian GABA transporter which comprises inserting nucleic acid encoding the mammalian GABA transporter in a suitable vector, inserting the resulting

20 vector in a suitable host cell, recovering the transporter produced by the resulting cell, and purifying the transporter so recovered. An example of an isolated GABA transporter is an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figures 1A or 1B. This invention

25 also provides a method of preparing the isolated mammalian taurine transporter which comprises inserting nucleic acid encoding a mammalian taurine transporter in a suitable vector, inserting the resulting vector in a suitable host cell, recovering the transporter produced

30 by the resulting cell, and purifying the transporter so recovered. This invention also provides a method of preparing the isolated human GABA transporter which comprises inserting nucleic acid encoding the human GABA transporter in a suitable vector, inserting the resulting

35 vector in a suitable host cell, recovering the human GABA

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transporter produced by the resulting cell, and purifying the human GABA transporter so recovered. These methods for preparing GABA or taurine transporters uses recombinant DNA technology methods well known in the art.

5 For example, isolated nucleic acid encoding GABA or taurine transporter is inserted in a suitable vector, such as an expression vector. A suitable host cell, such as a bacterial cell, or a eukaryotic cell such as a yeast cell, is transfected with the vector. GABA or  
10 taurine transporter is isolated from the culture medium by affinity purification or by chromatography or by other methods well known in the art.

15 This invention provides a method for determining whether a substrate not known to be capable of binding to a mammalian GABA transporter can bind to the mammalian GABA transporter which comprises contacting a mammalian cell comprising a DNA molecule encoding a mammalian GABA transporter with the substrate under conditions  
20 permitting binding of substrates known to bind to the transporter, detecting the presence of any of the substrate bound to the transporter, and thereby determining whether the substrate binds to the transporter. The DNA in the cell may have a coding  
25 sequence substantially the same as the coding sequences shown in Figures 1A, or 1B. This invention provides a method for determining whether a substrate not known to be capable of binding to a mammalian taurine transporter can bind to the mammalian GABA transporter which  
30 comprises contacting a mammalian cell comprising a DNA molecule encoding a mammalian taurine transporter with the substrate under conditions permitting binding of substrates known to bind to the transporter, detecting the presence of any of the substrate bound to the  
35 transporter, and thereby determining whether the

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substrate binds to the transporter. The DNA in the cell may have a coding sequence substantially the same as the coding sequences shown in Figure 1C.

5 This invention also provides a method for determining whether a substrate not known to be capable of binding to a human GABA transporter can bind to a human GABA transporter which comprises contacting a mammalian cell comprising a DNA molecule encoding a human GABA  
10 transporter with the substrate under conditions permitting binding of substrates known to bind to the transporter, detecting the presence of any of the substrate bound to the transporter, and thereby determining whether the substrate binds to the  
15 transporter. The DNA in the cell may have a coding sequence substantially the same as the coding sequences shown in Figures 10A or 10B. This invention also provides a method for determining whether a substrate not known to be capable of binding to a human taurine  
20 transporter can bind to a human taurine transporter which comprises contacting a mammalian cell comprising a DNA molecule encoding a human taurine transporter with the substrate under conditions permitting binding of substrates known to bind to the transporter, detecting  
25 the presence of any of the substrate bound to the transporter, and thereby determining whether the substrate binds to the transporter. Preferably, the mammalian cell is nonneuronal in origin. An example of a nonneuronal mammalian cell is a Cos7 cell. The  
30 preferred method for determining whether a substrate is capable of binding to the mammalian transporter comprises contacting a transfected nonneuronal mammalian cell (i.e. a cell that does not naturally express any type of transporter, thus will only express such a transporter if  
35 it is transfected into the cell) expressing a transporter



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on its surface, or contacting a membrane preparation derived from such a transfected cell, with the substrate under conditions which are known to prevail, and thus to be associated with, in vivo binding of the substrates to a transporter, detecting the presence of any of the substrate being tested bound to the transporter on the surface of the cell, and thereby determining whether the substrate binds to the transporter. This response system is obtained by transfection of isolated DNA into a suitable host cell. Such a host system might be isolated from pre-existing cell lines, or can be generated by inserting appropriate components into existing cell lines. Such a transfection system provides a complete response system for investigation or assay of the functional activity of mammalian transporters with substrates as described above. Transfection systems are useful as living cell cultures for competitive binding assays between known or candidate drugs and substrates which bind to the transporter and which are labeled by radioactive, spectroscopic or other reagents. Membrane preparations containing the transporter isolated from transfected cells are also useful for these competitive binding assays. A transfection system constitutes a "drug discovery system" useful for the identification of natural or synthetic compounds with potential for drug development that can be further modified or used directly as therapeutic compounds to activate or inhibit the natural functions of the mammalian transporter and/or the human transporter. The transfection system is also useful for determining the affinity and efficacy of known drugs at the mammalian transporter sites and human transporter sites.

This invention provides a method for isolating membranes which comprise GABA or taurine transporters. In a

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preferred embodiment of the invention, membranes comprising a GABA or taurine transporter are isolated from transfected cells comprising a plasmid vector which further comprises the regulatory elements necessary for the expression of the DNA encoding a GABA or taurine transporter so located relative to the DNA encoding the GABA or taurine transporter as to permit expression thereof. The DNA may have the coding sequence substantially the same as the sequence shown in Figure 1A, 1B, 1C, 10A or 10B. The host cell may be a bacterial, yeast, or a mammalian cell. Examples of such cells include the mouse fibroblast cell line NIH3T3, CHO cells, HELA cells, Ltk- cells and Y1 cells. A method for isolating membranes which contain a GABA or taurine transporter comprises preparing a cell lysate from cells expressing the GABA or taurine transporter and isolating membranes from the cell lysate. Methods for the isolation of membranes are well known by one of skill in the art. A method for the isolation of membranes from transfected cells is further described by Branchek et al. (1990). Membranes isolated from transfected cells expressing a GABA or taurine transporter are useful for identifying compounds which may include substrates, drugs or other molecules that specifically bind to a GABA or taurine transporter using radioligand binding methods (Branchek et al. 1990) or other methods described herein. The specificity of the binding of the compound to the transporter may be identified by its high affinity for a particular transporter.

This invention further provides a method for the isolation of vesicles from cells expressing a GABA or taurine transporter. In a preferred embodiment of the invention, vesicles comprising a GABA or taurine transporter are isolated from transfected cells

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comprising a plasmid vector which further comprises the regulatory elements necessary for the expression of the DNA encoding a GABA or taurine transporter so located relative to the DNA encoding the GABA or taurine transporter as to permit expression thereof. The DNA may have the coding sequence substantially the same as the sequence shown in Figure 1A, 1B, 1C, 10A or 10B. A method for the isolation of vesicles is described by Barber and Jamieson (1970) and by Mabjeesh et al. (1992). Vesicles comprising a GABA or taurine transporter are useful for assaying and identifying compounds, which may include substrates, drugs or other molecules that enhance or decrease GABA or taurine transporter activity. The compounds may modulate transporter activity by interacting directly with the transporter or by interacting with other cellular components that modulate transporter activity. Vesicles provide an advantage over whole cells in that the vesicles permit one to choose the ionic compositions on both sides of the membrane such that transporter activity and its modulation by can be studied under a variety of controlled physiological or non-physiological conditions. Methods for the assay of transporter activity are well known by one of skill in the art and are described herein below and by Kannner (1978) and Rudnick (1977).

This invention also provides a method of screening drugs to identify drugs which specifically interact with, and bind to, the mammalian GABA transporter on the surface of a cell which comprises contacting a mammalian cell comprising a DNA molecule encoding a mammalian GABA transporter on the surface of a cell with a plurality of drugs, detecting those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, the mammalian GABA

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transporter. The DNA in the cell may have a coding sequence substantially the same as the coding sequences shown in Figure 1A or 1B. This invention also provides a method of screening drugs to identify drugs which specifically interact with, and bind to, the mammalian taurine transporter on the surface of a cell which comprises contacting a mammalian cell comprising a DNA molecule encoding a mammalian taurine transporter on the surface of a cell with a plurality of drugs, detecting those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, the mammalian taurine transporter. The DNA in the cell may have a coding sequence substantially the same as the coding sequences shown in Figure 1C. This invention also provides a method of screening drugs to identify drugs which specifically interact with, and bind to, a human GABA transporter on the surface of a cell which comprises contacting a mammalian cell comprising a DNA molecule encoding a human GABA transporter on the surface of a cell with a plurality of drugs, detecting those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, the human GABA transporter. The DNA in the cell may have a coding sequence substantially the same as the coding sequences shown in Figures 10A or 10B. This invention also provides a method of screening drugs to identify drugs which specifically interact with, and bind to, a human taurine transporter on the surface of a cell which comprises contacting a mammalian cell comprising a DNA molecule encoding a human taurine transporter on the surface of a cell with a plurality of drugs, detecting those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, the human taurine transporter. Various methods of detection may be employed. The drugs may be "labeled"

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by association with a detectable marker substance (e.g., radiolabel or a non-isotopic label such as biotin). Preferably, the mammalian cell is nonneuronal in origin. An example of a nonneuronal mammalian cell is a Cos7 cell. Drug candidates are identified by choosing chemical compounds which bind with high affinity to the expressed transporter protein in transfected cells, using radioligand binding methods well known in the art, examples of which are shown in the binding assays described herein. Drug candidates are also screened for selectivity by identifying compounds which bind with high affinity to one particular transporter subtype but do not bind with high affinity to any other transporter subtype or to any other known transporter site. Because selective, high affinity compounds interact primarily with the target transporter site after administration to the patient, the chances of producing a drug with unwanted side effects are minimized by this approach. This invention provides a pharmaceutical composition comprising a drug identified by the method described above and a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. Once the candidate drug has been shown to be adequately bio-available following a particular route of administration, for example orally or by injection (adequate therapeutic concentrations must be maintained at the site of action for an adequate period to gain the desired therapeutic benefit), and has been shown to be non-toxic and therapeutically effective in appropriate disease models, the drug may be administered to patients by that route of administration determined to make the drug bio-available,

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in an appropriate solid or solution formulation, to gain the desired therapeutic benefit.

Applicants have identified individual transporter subtype proteins and have described methods for the identification of pharmacological compounds for therapeutic treatments. Pharmacological compounds which are directed against specific transporter subtypes provide effective new therapies with minimal side effects.

Elucidation of the molecular structures of the neuronal GABA and taurine transporters is an important step in the understanding of GABAergic neurotransmission. This disclosure reports the isolation, amino acid sequence, and functional expression of a cDNA clones from rat brain which encode a GABA transporters and a cDNA clone from rat brain which encodes a taurine transporter. This disclosure reports the isolation, amino acid sequence, and functional expression of cDNA clones which encode human GABA transporters. The identification of these transporters will play a pivotal role in elucidating the molecular mechanisms underlying GABAergic transmission, and should also aid in the development of novel therapeutic agents.

Complementary DNA clones (designated rB14b, rB8b, and rB16a) encoding two GABA transporters and a taurine transporter, respectively, have been isolated from rat brain, and their functional properties have been examined in mammalian cells. The nucleotide sequence of rB14b predicts a protein of 602 amino acids, rB8b predicts a protein of 627 amino acids, and rB16a predicts a protein of 621 amino acids, with 12 highly hydrophobic regions compatible with membrane-spanning domains. When

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incubated with 50 nM [<sup>3</sup>H]GABA, COS cells transiently transfected with rB14b or rB8b accumulated greater than 50-fold as much radioactivity as non-transfected control cells. The transporters encoded by rB14b and rB8b display high-affinity for GABA ( $K_m=4\mu M$ ) and are dependent on external sodium and chloride. Similarly, when incubated with 50nM [<sup>3</sup>H]taurine, Cos cells transiently transfected with rB21a accumulated approximately 7-fold as much radioactivity as non-transfected control cells. The pattern of expression of mRNA encoding two GABA transporters has been examined in the rat brain. Additionally, complementary DNA clones (designated hGAT-3, hHE7a, hS3a) and a genomic DNA clone encoding human GABA transporters have been isolated and their functional properties examined in mammalian cells.

Analysis of the GABA and taurine transporter structure and function provides a model for the development of drugs useful for the treatment of epilepsy, generalized anxiety, migraine, ischemia and other neurological disorders.

This invention identifies for the first time three new mammalian transporter proteins, their amino acid sequences, and their mammalian genes. The invention further identifies the human homologues of two mammalian GABA transporter proteins, their amino acid sequence and their human genes. The information and experimental tools provided by this discovery are useful to generate new therapeutic agents, and new therapeutic or diagnostic assays for these new transporter proteins, their associated mRNA molecules or their associated genomic DNAs. The information and experimental tools provided by this discovery will be useful to generate new therapeutic agents, and new therapeutic or diagnostic assays for

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these new transporter proteins, their associated mRNA molecules, or their associated genomic DNAs.

Specifically, this invention relates to the first  
5 isolation of three mammalian cDNAs and genomic clones  
encoding GABA and taurine transporters and the first  
isolation of cDNAs and a genomic clone encoding the human  
homologues of two mammalian GABA transporters. The new  
mammalian genes for these transporters identified herein  
10 as rB14b, rB8b, and rB16a have been identified and  
characterized, and a series of related cDNA and genomic  
clones have been isolated. In addition, the mammalian  
GABA and mammalian taurine transporters have been  
expressed in Cos7 cells by transfecting the cells with  
15 the plasmids EVJB-rB14b, EVJB-rB8b, and EVJB-rB16a. The  
pharmacological binding properties of the proteins  
encoded have been determined, and these binding  
properties classify these proteins as GABA transporters  
and a taurine transporter. Mammalian cell lines  
20 expressing the mammalian and human GABA transporters and  
the mammalian taurine transporter on the cell surface  
have been constructed, thus establishing the first  
well-defined, cultured cell lines with which to study the  
GABA and taurine transporters.

25 This invention will be better understood by reference to  
the Experimental Details which follow, but those skilled  
in the art will readily appreciate that the specific  
experiments detailed are only illustrative, and are not  
30 meant to limit the invention as described herein, which  
is defined by the claims which follow thereafter.

#### MATERIALS and METHODS



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**Materials for Mammalian GABA Transporter Studies:**

[<sup>3</sup>H]GABA<sup>3</sup> (98.9Ci/mmol) was obtained from New England Nuclear (Boston, MA).  $\beta$ -alanine, betaine and L-DABA (L-(2,4) diaminobutyric acid) were from Sigma Chemical Company (St. Louis, MO); guvacine, nipecotic acid, OH-nipecotic (hydroxynipecotic acid), and THPO (4,5,6,7-tetrahydroisoxazolo (4,5-c]pyridin-3-ol) were from RBI (Natick, MA). ACHC (cis-3-aminocyclohexanecarboxylic acid) was kindly provided by Drs. Richard Milius and William White of the NIMH Chemical Synthesis Program.

**Materials for Mammalian Taurine Transporter Studies:**

[<sup>3</sup>H]taurine (25.6Ci/mmol) was from New England Nuclear (Boston, MA); taurine, GABA<sup>2</sup>, hypotaurine, AEPA, AMSA, APSA, CSA, MEA, and  $\beta$ -alanine were from Sigma Chemical Corporation (St. Louis, MO); GES was a kind gift of Dr. J. Barry Lombardini (Department of Pharmacology, Texas Tech University).

**Cloning and Sequencing of Mammalian GABA Transporters:** A rat brain cDNA library in the Lambda ZAP II vector (Stratagene, La Jolla, CA) was screened at reduced stringency using probes representing the complete coding region of the rat GABA transporter cDNA (GAT-1 (21)). Exact primers derived from the nucleotide sequence of GAT-1 were used to generate GAT-1 PCR products from randomly-primed rat brain cDNA; the GAT-1 probes were then labeled and used to screen the library under reduced stringency as previously described (68). Lambda phage hybridizing with the probes at low stringency were plaque purified and rescreened at high stringency to eliminate clones which were identical to GAT-1. One of the clones hybridizing at high stringency was subsequently confirmed by sequence analysis to encode GAT-1 (21). Clones hybridizing only at low stringency were converted to

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phagemids by in vivo excision with f1 helper phage. Nucleotide sequences of double-stranded cDNAs in pBluescript were analyzed by the Sanger dideoxy nucleotide chain-termination method (59) using Sequence  
5 (U.S. Biochemical Corp., Cleveland, Ohio).

**Expression of Mammalian GABA Transporters:** cDNA clones (designated rB14b and rB8b) representing the complete coding regions of two putative transporters were cloned  
10 into the eukaryotic expression vector pEVJB (modified from pcEXV-3; (51)). Utilizing restriction enzyme sites present in pBluescript, rB14b was subcloned as a 2.0 kb HindIII/XbaI fragment which contained 126 base pairs of 5'-untranslated sequence and 94 base pairs of 3'-  
15 untranslated sequence. Similarly, rB8b was subcloned as a 2.1 kb XbaI/SalI fragment containing 0.3 kb of 3'-untranslated sequence. Transient transfections of COS cells were carried out using DEAE-dextran with DMSO according to the method of Lopata et al. (44) with minor  
20 modifications. COS cells were grown (37°C., 5%CO<sub>2</sub>) in high glucose Dulbecco's modified Eagle medium supplemented with 10% bovine calf serum, 100 U/ml penicillin G, and 100 µg/ml streptomycin sulfate. Cells were routinely used two days after transfection for  
25 transport studies.

**Transport Studies of Mammalian GABA Transporters:**  
To measure transport, COS cells grown in 6-well (well diameter = 35mm) or 24-well (well diameter = 18mm) plates  
30 were washed 3X with HEPES-buffered saline (HBS, in mM: NaCl, 150; HEPES, 20; CaCl<sub>2</sub>, 1; glucose, 10; KCl, 5; MgCl<sub>2</sub>, 1; pH 7.4) and allowed to equilibrate in a 37°C water bath. After 10 minutes the medium was removed and a solution containing [<sup>3</sup>H]GABA (New England Nuclear, sp.  
35 activity = 89.8Ci/mmol) and required drugs in HBS was

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added (1.5 ml/35mm well; 0.5ml/18mm well). Non-specific uptake was defined in parallel wells with 1mM unlabeled substrate, and was subtracted from total uptake (no competitor) to yield specific uptake; all data represent specific uptake. Plates were incubated at 37°C for 10 minutes unless indicated otherwise, then washed rapidly 3x with ice-cold HBS. Cells were solubilized with 0.05% sodium deoxycholate/0.1N NaOH, an aliquot neutralized with 1N HCl, and radioactivity was determined by scintillation counting. Protein was quantified in an aliquot of the solubilized cells using a BIO-RAD protein assay kit, according to the manufacturers directions.

#### Northern Blot Analysis of RNA Encoding Mammalian Transporters:

Total cellular RNA was isolated from rat brain and liver using RNazol (Cinna/Biotech Laboratories Inc.; Houston, TX) as outlined by the manufacturer. Denatured RNA samples (25µg) were separated in a 1.0% agarose gel containing 3.3% formaldehyde. RNAs were transferred to nylon membranes (Genescreen Plus; New England Nuclear, Boston, MA) by overnight capillary blotting in 10X SSC. Northern blots were rinsed and then baked for 2 hours at 80°C under vacuum. Prehybridization was for 2 hours at 65°C in a solution containing 50% formamide, 1M NaCl, 10% dextran sulfate, and 1% sodium dodecyl sulfate. Blots were hybridized overnight at 65°C with <sup>32</sup>P-labeled DNA probes (randomly primed GAT-2 or GAT-3 full-length cDNA clones) in prehybridization mixture containing 100 µg/ml sonicated salmon sperm DNA. The blots were washed successively in 2X SSC/2% SDS, 1X SSC/2% SDS, and 0.2X SSC/2% SDS at 65°C, then exposed to Kodak XAR-5 film with one intensifying screen at -90°C for four days.

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**Tissue Localization Studies:** To identify tissues expressing mRNAs for the novel GABA transporters and the previously cloned GABA transporter GAT-1 (21), specific PCR primers (25mers) were designed such that  $\approx 700$  base pair fragments encoding TMs 1 through 5 of each transporter could be amplified and detected by hybridization with  $^{32}\text{P}$ -labeled oligonucleotides. For rB14b, the sequences of the sense and anti-sense oligonucleotides were derived from amino acids 36 to 43 (5'-GACCAACAAGATGGAGTTCGTACTG) and 247 to 254 (5'-TGTTACTCCTCGGATCAACAGGACC); for rB8b, the oligonucleotides were derived from amino acids 52 to 60 (5'-GGAGTTCGTGTTGAGCGTAGGAGAG) and 271 to 279 (5'-GAACTTGATGCCTTCCGAGGCACCC); and for GAT-1 (21), the oligonucleotide sequences were derived from amino acids 50 to 57 (5'-ACGCTTCGACTTCCTCATGTCCTGT) and 274 to 282 (5'-GAATCAGACAGCTTTCGGAAGTTGG). Primers were also designed to amplify the cDNA encoding cyclophilin, a constitutively expressed gene, as a control (5'-GTCTGCTTCGAGCTGTTTGCAGACA, sense; 5'-TTAGAGTTGTCCACAGTCGGAGATG, anti-sense) (12). To detect amplified sequences, oligonucleotide probes were synthesized for GAT-1, rB14b, and rB8b which corresponded to amino acids 196 to 219, 161 to 183, and 207 to 229, respectively. Each probe was shown to hybridize with its respective transporter cDNA and not with any other transporter cDNA under study.

Poly A+ RNA (1  $\mu\text{g}$ , Clontech, Palo Alto, CA) from each of seven rat tissues was converted to single-stranded cDNA by random priming using Superscript reverse transcriptase (BRL, Gaithersburg, MD). PCR reactions were carried out in a buffer containing 20mM Tris (pH 8.3), 50 mM KCl, 1.5mM  $\text{MgCl}_2$ , 0.001% gelatin, 2mM dNTP's, 1 $\mu\text{M}$  each primer, and Taq polymerase with either cDNA, RNA, water, or a

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control plasmid for 30 cycles of 94°C./2 min., 68°C./2 min., 72°C./3 min. PCR products were separated by electrophoresis in 1.2% agarose gels, blotted to nylon membranes (Genescreen Plus; New England Nuclear, Boston, MA), and hybridized at 40°C. overnight with <sup>32</sup>P-labeled oligonucleotide probes in a solution containing 50% formamide, 10% dextran sulfate, 5X SSC, 1X Denhardt's, and 100 µg/ml sonicated salmon sperm DNA. Blots were washed successively in 2 X SSC at room temperature and 0.1 X SSC at 50°C., and exposed to Kodak XAR film for 0.5 to 4 hours with an intensifying screen at -70°C.

**Cloning and Sequencing of Mammalian Taurine Receptor:** A rat brain cDNA library in the Lambda ZAP II vector (Stratagene, La Jolla, CA) was screened at low stringency with the complete coding region of the rat GABA transporter cDNA (GAT-1; (21)). Exact primers were used to generate PCR products from randomly-primed rat brain cDNA; the products were labeled and used to screen the library under reduced stringency (25% formamide, 40°C. hybridization; 0.1X SSC, 40°C. wash) as previously described (68). Lambda phage hybridizing at low stringency with the GAT-1 sequence were plaque purified and rescreened with the same probes at high stringency (50% formamide, 40°C. hybridization; 0.1X SSC, 50°C. wash) to eliminate clones identical to GAT-1. Clones hybridizing only at low stringency were converted to phagemids by in vivo excision with f1 helper phage. Nucleotide sequences of double-stranded cDNAs in pBluescript were analyzed by the Sanger dideoxy nucleotide chain-termination method (59) using Sequenase (U.S. Biochemical Corp., Cleveland, Ohio).

**Expression of Mammalian Taurine Transporter:** A complementary DNA (designated rB16a) containing the

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complete coding region of a putative transporter was cloned into the eukaryotic expression vector pEVJB (modified from pcEXV-3; (51)) as a 2.5 kb XbaI/SalI fragment using restriction enzyme sites within the vector. In addition to the coding region, 0.1 kb of 5'-untranslated sequence and 0.5 kb of 3'-untranslated sequence were included in the construct. Transient transfections of COS cells with the plasmid pEVJB-rB16a were carried out using DEAE-dextran with DMSO according to the method of Lopata et al. (44) with minor modifications. COS cells were grown (37°C, 5%CO<sub>2</sub>) in high glucose Dulbecco's modified Eagle medium supplemented with 10% bovine calf serum, 100 U/ml penicillin G, and 100 µg/ml streptomycin sulfate. Cells were routinely used two days after transfection for transport studies.

**Transport Studies of Mammalian Taurine Transporter:** To measure transport, COS cells grown in 6-well (well diameter = 35mm) or 24-well (well diameter = 18mm) plates were washed 3X with HEPES-buffered saline (HBS, in mM: NaCl, 150; HEPES, 20; CaCl<sub>2</sub>, 1; glucose, 10; KCl, 5; MgCl<sub>2</sub>, 1; pH 7.4) and allowed to equilibrate in a 37°C water bath. After 10 minutes the medium was removed and a solution containing [<sup>3</sup>H]taurine (New England Nuclear, sp. activity = 25.6 Ci/mmol) and required drugs in HBS was added (1.5 ml/35mm well; 0.5ml/18mm well). Non-specific uptake was defined in parallel wells with 1mM unlabeled taurine and was subtracted from total uptake (no competitor) to yield specific uptake; all data represent specific uptake. Plates were incubated at 37°C for 10 minutes unless indicated otherwise, then washed rapidly 3X with ice-cold HBS. Cells were solubilized with 0.05% sodium deoxycholate/0.1N NaOH, an aliquot was neutralized with 1N HCl, and radioactivity was determined by scintillation counting. Protein was quantified in an

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aliquot of the solubilized cells using a BIO-RAD protein assay kit, according to the manufacturer's directions.

PCR Tissue Localization Studies of Mammalian Taurine  
5     Transporter: To identify tissues expressing mRNA for the  
taurine transporter, exact primers (25mers) were designed  
such that a 707 base pair fragment of rB16a could be  
amplified from cDNA and detected by Southern blot  
analysis. The sequences of the sense and anti-sense  
10     primers were derived from amino acids 40 to 47 (5'-  
TCAGAGGGAGAAGTGGTCCAGCAAG) and 268 to 275 (5'-  
ATTTCATGCCTTCACCAGCACCTGG), respectively. Primers were  
also designed to amplify the cDNA encoding cyclophilin  
(12), a constitutively expressed gene, as control (5'-  
15     ACGCTTCGACTTCCTCATGTCCTGT, sense; 5'-  
TTAGAGTTGTCCACAGTCGGAGATG, antisense). To detect  
amplified sequences, an oligonucleotide probe was  
synthesized (corresponding to amino acids 249 to 271)  
which was specific for rB16a. Poly A+ RNA (1 µg,  
20     Clontech, Palo Alto, CA) from each of seven rat tissues  
was converted to single-stranded cDNA by random priming  
using Superscript reverse transcriptase (BRL,  
Gaithersburg, MD). PCR reactions were carried out in a  
buffer containing 20mM Tris (pH 8.3), 50 mM KCl, 1.5mM  
25     MgCl<sub>2</sub>, 0.001% gelatin, 2mM dNTP's, 1µM each primer, Taq  
polymerase, and either cDNA, RNA, water, or a control  
plasmid containing rB16a for 30 cycles of 94°C./2 min.,  
68°C./2 min., 72°C./3 min. PCR products were separated  
by electrophoresis in 1.2% agarose gels, blotted to nylon  
30     membranes (Genescreen Plus; New England Nuclear, Boston,  
MA), and hybridized at 40°C. overnight with specific <sup>32</sup>P-  
labeled oligonucleotides in a solution containing 50%  
formamide, 10% dextran sulfate, 5X SSC, 1X Denhardt's,  
and 100 µg/ml of sonicated salmon sperm DNA. Blots were  
35     washed at high-stringency (0.1X SSC, 50°C.) and exposed

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to Kodak XAR film for 0.5 to 4 hours with one intensifying screen at -70°C.

5 Northern Blot Analysis of mRNA encoding Mammalian Taurine  
Transporter: Samples of poly A<sup>+</sup> RNA isolated from each  
of eight rat tissues (5 µg, Clontech; Palo Alto, CA) were  
separated in a 1.0% agarose gel containing 3.3%  
formaldehyde and transferred to a nylon membrane  
(Genescreen Plus; New England Nuclear, Boston, MA) by  
10 overnight capillary blotting in 10X SSC. Prior to  
hybridization, the Northern blot was incubated for 2  
hours at 42°C. in a solution containing 50% formamide, 1M  
NaCl, 10% dextran sulfate, and 1% sodium dodecyl sulfate  
(SDS). The blot was hybridized overnight at 42°C. with  
15 <sup>32</sup>P-labeled DNA probe (randomly-primed HindIII/KpnI  
fragment of rB16a representing amino acids 6-336) in the  
prehybridization solution containing 100 µg/ml sonicated  
salmon sperm DNA. The blot was washed successively in 2X  
SSC/2% SDS, 1X SSC/2% SDS, and 0.2X SSC/2% SDS at 65°C.  
20 and exposed to Kodak XAR-5 film with one intensifying  
screen at -70°C. for 1-4 days. To confirm that equal  
amounts of RNA were present in each lane, the same blot  
was rehybridized with a probe encoding cyclophilin (12).

25 Use of PCR to Identify human cDNA Libraries for  
Screening: For hGAT-2, the sequences of the rat PCR  
primers were 5'-GACCAACAAGATGGAGTT (sense) and 5'-  
TGTTACTCCTCGGATCAA (antisense). PCR reactions were  
carried out in a buffer containing 20mM Tris (pH 8.3), 50  
30 mM KCl, 1.5mM MgCl<sub>2</sub>, 0.001% gelatin, 2mM dNTP's, 1µM each  
primer, Taq polymerase, and an aliquot of a lambda phage  
library, water, or a control plasmid for 40 cycles of  
94°C. for 2 min., 50°C. for 2 min., and 72°C. for 3 min.  
For hGAT-3, the sequences of the degenerate primers were  
35 5'-TGGAATTCG(G/C)CAA(C/T)GTITGG(C/A)GITT(C/T)CCITA



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(sense) and 5'-TCGCGGCCGCAA(A/G)AAGATCTGIGTIGCIGC(A/G)TC (antisense). PCR reactions were carried out as described above for 40 cycles of 94°C. for 2 min., 40°C. for 2 min., and 72°C. for 3 min. PCR products were separated by electrophoresis in 1.2% agarose gels, blotted to nylon membranes (Genescreen Plus; New England Nuclear, Boston, MA), and hybridized at 40°C. overnight with <sup>32</sup>P-labeled probes in a solution containing 25% formamide, 10% dextran sulfate, 5X SSC, 1X Denhardt's, and 100 µg/ml of sonicated salmon sperm DNA. Blots were washed at low stringency (0.1X SSC, 40°C.) and exposed to Kodak XAR film for up to three days with one intensifying screen at -70°C.

**Isolation and Sequencing of Human Clones:** Human cDNA libraries in the Lambda ZAP II vector (Stratagene, La Jolla, CA) that were identified as containing hGAT-2 or hGAT-3 were screened under either reduced stringency (25% formamide, 40°C. hybridization; 0.1X SSC, 40°C. wash) or high stringency (50% formamide, 40°C. hybridization; 0.1X SSC, 50°C. wash). Hybridizing lambda phage were plaque purified and converted to phagemids by in vivo excision with f1 helper phage. Nucleotide sequences of double-stranded cDNAs in pBluescript were analyzed by the Sanger dideoxy nucleotide chain-termination method (59) using Sequenase (U.S. Biochemical Corp., Cleveland, Ohio). Fragments of genomic clones in the lambda FIX II vector were subcloned into pUC18 prior to double-stranded sequencing.

**Preparation of Primary Brain Cell Cultures:** Astrocytes, neurons and meningeal fibroblasts were prepared from the brains of E19 embryonic rats. Briefly, the brains were removed, dissected free of meninges, and trypsinized. Cells were dissociated mechanically by passage through a

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Pasteur pipet, and resuspended in DMEM containing 10% fetal bovine serum and antibiotics. The cells were added to tissue culture dishes that had been previously coated with 10 $\mu$ M poly-D-lysine.

5

For astrocytes, the cells were plated at a density of approximately 3x10<sup>6</sup> cells per 100mm dish. The astrocytes were allowed to reach confluence, then passaged 1 or 2 times prior to harvesting. For neurons, a plating density of 15x10<sup>6</sup> cells per 100mm dish was employed; the medium was supplemented with insulin. Cytosine arabinoside (ara-C) was added to a final concentration of 10 $\mu$ M on day 2 or 3 to inhibit the proliferation of non-neuronal cells. The neurons were harvested 1 week after plating. To obtain meningeal fibroblasts the meninges were trypsinized, then mechanically dissociated as described above. The cells recovered from a single embryo were plated into a 100mm dish, grown to confluence, and passaged 1-2 times prior to harvesting.

20

**Isolation of RNA from Cell Cultures:** Plates were placed on ice and quickly rinsed twice with ice-cold phosphate-buffered saline (PBS). Cells were then dissolved in 10mls lysis solution (7M urea, 350mM NaCl, 2% sodium dodecyl sulfate (SDS), 1mM EDTA, and 10 mM Tris-HCl, pH 8.0) and transferred to a sterile tube. Lysates were homogenized (Virtis, lowest speed, 5 seconds) and then digested with proteinase K (0.1mg/ml) at 37°C. for 30 minutes. Samples were extracted twice with phenol/chloroform and once with chloroform before ethanol precipitation. Total RNA was collected by centrifugation, resuspended in diethylpyrocarbonate (DEPC)-treated water, and stored at -20°C. until use.

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**Detection of Transporter mRNAs using PCR:** To identify cell types expressing mRNAs for the GABA transporters GAT-1, GAT-2, and GAT-3, specific PCR primers (25mers) were designed such that  $\approx 700$  base pair fragments encoding transmembrane domains 1 through 5 of each transporter could be amplified and detected by hybridization with  $^{32}\text{P}$ -labeled oligonucleotides. For rB14b (GAT-2), the sequences of the sense and anti-sense oligonucleotides were derived from amino acids 36 to 43 (5'-GACCAACAAGATGGAGTTCGTACTG) and 247 to 254 (5'-TGTTACTCCTCGGATCAACAGGACC); for rB8b (GAT-3), the oligonucleotides were derived from amino acids 52 to 60 (5'-GGAGTTCGTGTTGAGCGTAGGAGAG) and 271 to 279 (5'-GAACTTGATGCCTTCCGAGGCACCC); and for GAT-1 (21), the oligonucleotide sequences were derived from amino acids 50 to 57 (5'-ACGCTTCGACTTCCTCATGTCCTGT) and 274 to 282 (5'-GAATCAGACAGCTTTCGGAAGTTGG). To detect amplified sequences, oligonucleotide probes were synthesized for GAT-1, GAT-2, and GAT-3 which corresponded to amino acids 196 to 219, 161 to 183, and 207 to 229, respectively. Each probe was shown to hybridize with its respective transporter cDNA and not with the other transporter cDNAs.

Total RNA (0.5 $\mu\text{g}$ ) isolated from cultured neurons, astrocytes, and fibroblasts was converted to single-stranded cDNA by random priming using Superscript reverse transcriptase (BRL, Gaithersburg, MD). PCR reactions were carried out in a buffer containing 20mM Tris (pH 8.3), 50 mM KCl, 1.5mM  $\text{MgCl}_2$ , 0.001% gelatin, 2mM dNTP's, 1 $\mu\text{M}$  each primer, and Taq polymerase with either cDNA, RNA, water, or a control plasmid for 30 cycles of 94°C./2 min., 68°C./2 min., 72°C./3 min. PCR products were separated by electrophoresis in 1.2% agarose gels, blotted to nylon membranes (Genescreen Plus; New England

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Nuclear, Boston, MA), and hybridized at 40°C. overnight with <sup>32</sup>P-labeled oligonucleotide probes in a solution containing 50% formamide, 10% dextran sulfate, 5X SSC, 1X Denhardt's, and 100 µg/ml sonicated salmon sperm DNA.

5 Blots were washed successively in 2X SSC, 0.1% SDS at room temperature and 0.1X SSC, 0.1% SDS at 50°C., and exposed to Kodak XAR film for 0.5 to 4 hours with an intensifying screen at -70°C.

10 **In Situ Hybridization:** Male Sprague-Dawley rats (Charles River) were decapitated and the brains rapidly frozen in isopentane. Sections were cut on a cryostat, thaw-mounted onto poly-L-lysine coated coverslips, and stored at -80°C until use. Tissue was fixed in 4%  
15 paraformaldehyde, treated with 5mM dithiothreitol (DTT), acetylated (0.25% acetic anhydride in 0.1M triethanolamine), and dehydrated. Tissue was prehybridized (1 hour, 40°C) in a solution containing 50% formamide, 4X SSC (0.6M NaCl/0.06M sodium citrate), 1X  
20 Denhardt's solution (0.2% polyvinylpyrrolidone, 0.2% Ficoll, 0.2% bovine serum albumin), 50mM DTT, 500µg/ml salmon sperm DNA, 500µg/ml yeast tRNA, 10% dextran sulfate, then hybridized overnight with <sup>35</sup>S-labeled anti-sense oligonucleotides (45mers) in the same solution.  
25 After washing and dehydration, sections were apposed to Kodak X-OMAT AR film for 4 days at -20°C. To verify the specificity of the hybridization signal, parallel tissues were pretreated with 100 µg/ml RNase A (37°, 30 minutes) prior to hybridization. Two different oligonucleotides  
30 designed to separate regions of the GABA transporters (loop region between transmembrane domains III and IV, 3'untranslated region) showed identical patterns of hybridization.

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## 1. GABA Transporters

### RESULTS

#### Cloning of New Mammalian GABA Transporter Sequences:

5 We screened a rat brain cDNA library at low stringency with probes encoding the rat neuronal GABA transporter (GAT-1; (21)) in order to identify additional inhibitory amino acid transporter genes. Two clones were identified which hybridized at low but not at high stringency with  
10 the GABA transporter probes. DNA sequence analysis revealed that the clones encoded putative transporters which were structurally related to GAT-1. The first clone, rB14b, contained a 2.0 kb sequence with an open reading frame of 1806 base pairs which could encode a  
15 protein of 602 amino acids (Figure 1A). The second clone, rB8b, contained a 2.1 kb sequence which had an open reading frame of 1881 base pairs encoding a protein of 627 amino acids (Figure 1B). rB14b and rB8b exhibited 59% nucleotide identity throughout the coding region with  
20 the neuronal rat GABA transporter (GAT-1) and 70% nucleotide identity with each other. Comparison to sequences in Genbank and EMBL data bases demonstrated that both nucleotide sequences were novel and that the most homologous sequence was the rat GABA transporter  
25 GAT-1 (21). Subsequent comparisons which included recently cloned transporters revealed that the most closely related sequence is the canine betaine transporter (79) which exhibits 69% nucleotide identity with both rB14b and rB8b. The taurine transporter (66)  
30 and the glycine transporter (68) are also significantly related, exhibiting ~64% and ~56% nucleotide identity, respectively, to both rB14b and rB8b.

35 The amino acid sequence deduced from the nucleotide sequence of rB14b is shown in Figure 1D modeled after the

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proposed membrane topology of GAT-1 (21). Residues identical to those in rB8b are shaded and represent 67% amino acid identity between the two clones. The translation products of both rB14b and rB8b are predicted to have relative molecular masses of  $\approx 68,000$  Daltons. Hydropathy analyses indicate the presence of 12 hydrophobic domains in both proteins which may represent membrane spanning segments. For each transporter, several potential sites for Asn-linked glycosylation are found in the extracellular loop between the third and fourth transmembrane domains. Comparison and alignment of the deduced amino acid sequences of rB14b (GAT-2) and rB8b (GAT-3) with the neuronal GABA transporter (GAT-1) (Figure 2) revealed 52.5% and 52% amino acid identities, respectively. The betaine transporter (Figure 2), which can also transport GABA (79) exhibited a significantly higher degree of homology-- 68% and 65% amino acid identities to rB14b and rB8b, respectively. Similarly, the transporter for taurine (66), an inhibitory amino acid, is 61% homologous to both. In contrast, comparison of the new transporters with the rat glycine transporter (Figure 2 and Ref.(68)) or the human norepinephrine transporter (55) showed a lower degree of amino acid identity (43-45%), similar to that between the neuronal GABA and norepinephrine transporters (46%). These data suggested that the new sequences might encode additional amino acid transporters expressed in the brain. To explore this possibility, the sequences were each placed in a mammalian expression vector, transfected into COS cells, and screened for transport of a variety of radiolabeled neurotransmitters and amino acids. These studies revealed (see below) that rB14b and rB8b encode novel GABA transporters with pharmacological properties distinct from the neuronal GABA transporter.

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Pharmacological Characterization of Mammalian GABA Transporters:

COS cells transiently transfected with rB14b or rB8b (COS/rB14b and COS/rB8b, respectively) accumulated more [<sup>3</sup>H]GABA than non-transfected control cells; representative experiments are shown in Figure 3. During a 10 minute incubation (37°C) with a low concentration of [<sup>3</sup>H]GABA, specific uptake was increased 52±11-fold (mean±SEM, n=6) and 64±12-fold (n=5) over control for rB14b and rB8b, respectively. In contrast, the uptake of [<sup>3</sup>H]glutamate, [<sup>3</sup>H]glycine, [<sup>3</sup>H]5-HT, [<sup>3</sup>H]dopamine, and [<sup>3</sup>H]taurine was unaltered. Specific uptake represented greater than 95% of total uptake in transfected cells. Uptake of [<sup>3</sup>H]GABA was not observed following mock transfection or transfection with an irrelevant insert, indicating that the enhanced uptake was not the result of non-specific perturbation of the membrane. The transport of [<sup>3</sup>H]GABA by both COS/rB14b and COS/rB8b was decreased >95% when Na<sup>+</sup> was replaced by Li<sup>+</sup> (Table 1); similar results were obtained with COS cells expressing GAT-1 (COS/GAT-1), which we re-cloned (see Materials and Methods). When Cl<sup>-</sup> was replaced by acetate, [<sup>3</sup>H]GABA transport by COS/GAT-1 was nearly completely eliminated (Table 1), consistent with previous results obtained with this transporter (21,29). In contrast, transport by COS/rB14b and COS/rB8b was decreased to 43 and 20% of control, respectively (Table 1). The difference in sensitivity to removal of chloride exhibited by the three transporters was statistically significant (GAT-1 vs. COS/rB14b, p<0.001; GAT-1 vs. rB8b, p<0.05; rB14b vs. rB8b, p<0.05).

To determine the affinity of GABA for the cloned transporters, COS/rB14b and COS/rB8b were incubated with various concentrations of [<sup>3</sup>H]GABA and the specific

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accumulation of radioactivity was determined. Accumulation of [ $^3\text{H}$ ]GABA was dose-dependent and reached saturation at higher concentrations (Figure 4). Non-linear regression analysis of the data yielded the following values:  $K_M = 8 \pm 3 \mu\text{M}$  and  $12 \pm 6 \mu\text{M}$ , and  $V_{\text{MAX}} = 2.5 \pm 1.2$  and  $3.0 \pm 0.9$  nmoles/mg protein for COS/rB14b and COS/rB8b, respectively (mean  $\pm$  SEM,  $n=4$  experiments). Taken together, these data indicate that both rB14b and rB8b encode saturable, high-affinity, sodium- and chloride-dependent GABA transporters. Accordingly, we propose the terms GAT-2 and GAT-3 for the transporters encoded by rB14b and rB8b, respectively, according to the nomenclature proposed by Guastella et al. (21).

To determine the pharmacological properties of the cloned GABA transporters, we examined the ability of various drugs to inhibit the accumulation of [ $^3\text{H}$ ]GABA by GAT-2 and GAT-3; for comparison, we also examined the pharmacology of GAT-1. As shown in Table 2, the pharmacological properties of GAT-2 and GAT-3 are similar to one another, but differ considerably from GAT-1. For example,  $\beta$ -alanine, a ligand reported to be selective for glial GABA transport (36), is more potent at the new cloned transporters than at GAT-1. In contrast, ACHC, guvacine, nipecotic acid, and hydroxynipecotic acid are more potent at GAT-1 than at GAT-2 and GAT-3. Interestingly, the two newly cloned transporters can be distinguished by L-DABA which displays high affinity for GAT-2 as well as GAT-1, but is less potent at GAT-3.

To further characterize the pharmacological properties of GAT-2 and GAT-3, we examined the ability of (R)-Tiagabine and CI-966 to inhibit the uptake of [ $^3\text{H}$ ]GABA; for comparison, we also examined these compounds at GAT-1. These compounds are lipophilic derivatives of



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nipecotic acid and guvacine, respectively. As shown in Table 2, (R)-Tiagabine at a concentration of 100 $\mu$ M completely inhibits uptake at GAT-1 but has no effect at GAT-2 and GAT-3. Tiagabine is reported to have high potency at both neuronal and glial GABA transporters (6), and has demonstrated efficacy as an anticonvulsant in early clinical trials (8). The finding that Tiagabine has very low affinity for GAT-2 and GAT-3 underscores the potential of these transporters as unique drug targets. Similar to Tiagabine, the GABA uptake blocker CI-966 (72) displays far greater potency at GAT-1 than at GAT-2 and GAT-3 (Table 2). CI-966 was developed as an anticonvulsant but was withdrawn due to severe side effects observed in Phase 1 clinical trials (63).

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Table 1. Ion Dependence of [ $^3\text{H}$ ]GABA Uptake

5	Condition <sup>a</sup>	<u>Uptake<sup>a</sup></u>		
		GAT-1	GAT-2	GAT-3
	Na <sup>+</sup> -free	0.5±0.3 (3)	0.1±0.06 (3)	0.3±0.03 (3)
	Cl <sup>-</sup> -free	5±2 (3)	43.2±4.0 (5)	20.2±5.8 (5)

10

<sup>a</sup>COS-7 cells transfected with rB46a, rB14b, or rB8b were incubated for 10 minutes (37°C) with 50nM [ $^3\text{H}$ ]GABA in either HBS, or in HBS in which Li<sup>+</sup> was substituted for Na<sup>+</sup> (Na<sup>+</sup>-free), or in which acetate was substituted for Cl<sup>-</sup> (Cl<sup>-</sup>-free). Non-specific uptake was determined with 1mM

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GABA. Data represent specific uptake, expressed as percent of uptake in HBS (mean ±SEM; values in parentheses indicate number of experiments).

Table 2. Pharmacological Specificity of [<sup>3</sup>H]GABA Uptake

5	Inhibitor <sup>a</sup>	concentration	<u>% Inhibition<sup>a</sup></u>		
			GAT-1	GAT-2	GAT-3
10	ACHC <sup>b</sup>	100μM	49±10(3)	3±3(3)	0±0(3)
	β-alanine	100μM	11±1(8)	86±1(8)	70±1(7)
	betaine	500μM	0(2)	9(2)	1(2)
	L-DABA	100μM	49±8(7)	43±8(7)	4±1(5)
	guvacine	10μM	41±3(4)	13±1(3)	8±5(3)
	OH-nipecotic	10μM	34±5(3)	9±7(3)	5±2(3)
	nipecotic	10μM	51±5(3)	5±5(3)	12±6(3)
15	THPO	100μM	10(2)	9(2)	4(2)
	(R)-Tiagabine	100μM	100±1(3)	0±1(3)	0±1(3)
	CI-966	100μM	91±2(3)	9±6(3)	10±6(3)

20 <sup>a</sup>COS-7 cells transfected with rB46a, rB14b, or rB8b were incubated for 10 minutes (37°C) with 50nM [<sup>3</sup>H]GABA and the indicated compounds. Non-specific uptake was determined with 1mM GABA. Data show percent displacement of specific [<sup>3</sup>H]GABA uptake, mean ±SEM (values in parentheses indicate number of experiments).

25

<sup>b</sup> L-DABA = L-(2,4)diaminobutyric acid

THPO = 4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridin-3-ol

ACHC = cis-3-aminocyclohexanecarboxylic acid

30 C I - 9 6 6 = [ 1 - [ 2 - [ b i s 4 -  
(trifluoromethyl)phenyl]methoxy]ethyl]- 1 , 2 , 5 , 6 -  
tetrahydro-3-pyridinecarboxylic acid

Tiagabine = (R)-N-[4,4-bis(3-methyl-2-thienyl)but-3-en-1-yl]nipecotic acid

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Tissue Localization Studies of Mammalian GABA Transporters:

To define the tissue distribution patterns of the novel GABA transporters, polymerase chain reaction (PCR) was used to detect each sequence in cDNA from seven different rat tissues. For comparison, the distribution of GAT-1 was also studied. Radiolabeled probes were used to detect individual PCR products by hybridization; each of the probes was highly specific for the transporter under study (data not shown). As shown in Figure 5B, GAT-1 was detectable in brain and retina but not liver, kidney, heart, spleen, or pancreas after 30 cycles of PCR. GAT-2 was present not only in brain and retina, but also in liver, kidney, and heart. Levels of GAT-2 mRNA were also detectable in spleen with overexposure of the autoradiogram (data not shown). Similar to GAT-1, the distribution of GAT-3 was limited to brain and retina. Cyclophilin was amplified to a similar extent from all the tissues (data not shown), indicating that adequate cDNA was present in each sample. Samples of poly A<sup>+</sup> RNA not treated with reverse transcriptase and subjected to identical PCR conditions showed no hybridization with the transporter probes (not shown), indicating that the signals obtained with cDNA could not be accounted for by genomic DNA contamination. Thus, among the tissues examined, the distribution of GAT-3 is limited to the CNS, while GAT-2 has a wide peripheral distribution as well. These results are supported by Northern blot analyses of total RNA isolated from rat brain and liver; a single  $\approx$ 2.4kb transcript hybridizing with GAT-2 is present in both liver and brain, while a  $\approx$ 4.7kb transcript hybridizing with GAT-3 is detectable only in brain (Figure 5A).

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**Cellular Localization of GABA Transporter mRNAs:**

Prior to the recent cloning of GABA transporters (4,21), pharmacological evidence suggested that multiple transporters contributed to the high-affinity GABA uptake observed in rat brain (30). Both neuronal and glial elements transport GABA, and preparations enriched in each cell type display differential sensitivities to inhibitors of GABA transport (5, 53, 61), suggesting the presence of distinct neuronal and glial GABA transporters. The ability to design neuronal- or glial- selective GABA uptake inhibitors would be a major advantage in the design of effective therapeutic agents. The GABA transporter cloned from rat brain, designated GAT-1 (21), displays a pharmacological profile consistent with a "neuronal"-type carrier. Our cloning of two additional GABA transporters from rat brain, GAT-2 and GAT-3 (previously termed Ggab1 and Ggaba2, respectively), confirms the principle of heterogeneity in high-affinity GABA transporters. Further, the sensitivity of GAT-2 and GAT-3 to inhibition by  $\beta$ -alanine distinguishes them from GAT-1, and raises the possibility that one or both represent "glial"-type transporters. The availability of three cloned high-affinity GABA transporters now provides the opportunity to begin to examine the relationship between the pharmacologically defined neuronal and glial subtypes, and the transporters encoded by the cloned genes.

The presence of mRNAs representing each of the three GABA transporters was investigated in primary cultures of embryonic rat brain neurons, astrocytes, and meningeal fibroblasts. Polymerase chain reaction (PCR) was used to amplify each sequence for detection with specific probes. As shown in Table 3, the messenger RNAs encoding each GABA transporter had a unique pattern of distribution.

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GAT-1 mRNA was present in all three culture types, whereas GAT-3 mRNA was restricted to neuronal cultures. GAT-2 mRNA was present in both astrocyte and fibroblast cultures, but not in neuronal cultures. Thus, GAT-2 and GAT-3, which exhibit extremely similar pharmacological profiles, display non-overlapping cellular distribution patterns. GAT-1, which displays a "neuronal"-type pharmacology, is apparently not restricted to a neuronal distribution.

Table 3. Cellular Localization of GABA Transporters by PCR.

	Neuronal Cultures	Astrocyte Cultures	Fibroblast Cultures
GAT-1	+	+	+
GAT-2	-	+	+
GAT-3	+	-	-

Total RNA isolated from cultured embryonic rat neurons, astrocytes, or fibroblasts was converted to cDNA and subjected to PCR for detection of mRNAs encoding GAT-1, GAT-2, and GAT-3 as described in Experimental Procedures. Amplified products were separated on agarose gels, blotted to nylon membranes, and hybridized with radiolabeled oligonucleotides specific for each transporter cDNA. The blot was exposed to film and the autoradiogram developed after several hours. A (+) sign signifies that a positive signal was detected on the autoradiogram; a (-) signifies that no signal was detectable. The same results were observed in two independent experiments.

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It is important to note that primary cultures, while enriched for a specific population of cells, may contain a small proportion of additional cell types. The sensitivity of PCR is sufficient to amplify a sequence contributed by a small number of cells; therefore, an unequivocal assignment of neuronal vs. glial localization would require combined *in situ* hybridization/immunocytochemistry. However, the presence of GAT-3 mRNA only in neuronal cultures suggests that detection of GAT-1 mRNA in astrocyte cultures is not due to the presence of contaminating neurons, and that GAT-1 is probably present in astrocytes in addition to neurons. The presence of GAT-1 and GAT-2 in fibroblast as well as astrocyte cultures may be explained by our recent finding that meningeal fibroblast cultures contain a large proportion of astrocytes as defined by staining with antibodies to glial fibrillary acidic protein (GFAP) (data not shown); thus, GAT-1 and GAT-2 signals in meningeal fibroblasts probably result from contaminating astrocytes.

These studies suggest that multiple high-affinity GABA transporter subtypes are present in different functional compartments, with at least two subtypes present in neurons (GAT-1 and GAT-3) and in glia (GAT-1 and GAT-2). Further, they indicate that pharmacologic agents selective for each subtype may have different therapeutic applications.

#### 30    Localization of GAT-1 and GAT-3 mRNA by in situ Hybridization:

In situ hybridization of GAT-1 and GAT-3 was carried out using antisense probes to the 3' untranslated region and the 3,4 extracellular loop of each clone. Hybridization

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of sense probes (control) to the same regions were also studied.

5 GAT-1 mRNA was observed in all rat brain areas examined (Table 4). In the telencephalon, the highest levels were observed in the glomerular layer of the olfactory bulb, the orbital cortex, the lateral septal nucleus, the ventral pallidum, the globus pallidus, amygdaloid area, and layer 4 of the cerebral cortex. Moderate levels were  
10 observed in the islands of Calleja, the internal and external plexiform layers, and the piriform, retrosplenial, and cingulate cortices, as well as in all regions of the hippocampal formation.

15 In the diencephalon, the highest levels were found in the paraventricular and reticular thalamic nuclei, and in the dorsal lateral geniculate. Lower levels were seen in the reuniens and rhomboid thalamic nuclei. In the hypothalamus, moderate levels were seen in the  
20 suprachiasmatic and paraventricular nuclei, and in the medial preoptic area. Lower levels were seen in the supraoptic and anterior hypothalamic nuclei.

25 In the midbrain, high levels were seen in the substantia nigra (pars compacta and pars reticulata), median raphe, and the olivary pretectal nucleus. Lower levels were observed in the superior colliculus.

30 No label was seen in the pontine nuclei, nor in the cerebellar Purkinje cells.

GAT-3 mRNA was observed throughout the neuraxis (Table 5). Within the telencephalon, the highest levels were detected in the medial septal nucleus, the nucleus of the  
35 diagonal band, and the ventral pallidum. Lower levels



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were found in the amygdala and the shell of the nucleus accumbens. Low levels were observed in the hippocampus. No labeling above background was observed in the neocortex.

5

In the thalamus, many nuclear groups were labeled. The areas with the highest labeling were the xiphoid, paraventricular, and rhomboid nuclei, and the zona incerta. Lower levels were observed in the following nuclei: reuniens, reticular, medial and lateral ventral posterior, and the medial geniculate. In the hypothalamus, moderate labeling was found in the lateral and ventromedial regions. Lower levels were observed in the arcuate nucleus and median eminence.

15

In the midbrain, the highest levels were observed in the dorsal tegmentum.

20

In the metencephalon, the highest levels were found in the medial vestibular and deep cerebellar nuclei, and lower levels in the lateral superior olivary nucleus. No label was observed in the cerebellar cortex.

25

30

A comparison of the localization of GAT-1 and GAT-3 mRNAs indicates that both are widely distributed in the brain, and while GAT-1 is more abundant on a per cell basis, the two tend to have overlapping distributions. Notable exceptions are cortex and hippocampus which contain large numbers of neurons containing GAT-1 mRNA but few cells with GAT-3 mRNA. On the other hand, GAT-3 mRNA levels appear to be higher than GAT-1 in the superficial layers of the superior colliculus and in the deep cerebellar nuclei.

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Table 4. In situ localization of GAT-1 in the Rat CNS

Area <sup>1</sup>	Labeling <sup>2</sup>	
	Probe 191 <u>AS 3'UT</u>	Probe 179 <u>AS 3,4 loop</u>
5		
	<u>BREGMA 6.20mm</u>	
	mitral cells	-
10	glomerular layer	++
	ext.plexiform layer	+ $\frac{1}{2}$
	ant. olf nerve	+/-
	<u>BREGMA 5.20mm</u>	
	ext.plexiform layer	+
15	int.plexiform layer	+
	ant.comm.intrabulb	+/-
	AOM,D,V	+
	orbital cortex m,v,l	+ $\frac{1}{2}$
	frontal. cortex	+ $\frac{1}{2}$
20	<u>BREGMA 1.60mm</u>	
	tenia tecta	+
	lat.septal nucleus	+/-
	lat.septal interm.	$\frac{1}{2}$ +
	ICjM	+ $\frac{1}{2}$
25	caudate-putamen	+/-
	AcbSh	+ $\frac{1}{2}$
	AcbC	$\frac{1}{2}$ +
	vent.pallidum	+++
	olf.tubercle	-
30	ICj	+
	piriform ctx.	+
	cingulate ctx	+
	indusium griseum	+ $\frac{1}{2}$
	<u>BREGMA-1.40mm</u>	
35	retrosplen.ctx	$\frac{1}{2}$ +
	cortex I	+
	IV	++
	V	+
	reticular thal.nu.	+ $\frac{1}{2}$
40	globus pallidus	+++
	caudate-putamen	+
	ant.dor thal.nu.	-
	paraventr. thal. nu	+ $\frac{1}{2}$
	supraoptic nu.	$\frac{1}{2}$ +
45	suprachiasmatic nu.	+
	med.preoptic area	+ $\frac{1}{2}$

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Table 4 (continued)

5	Area <sup>1</sup>	Labeling <sup>2</sup>	
		Probe 191 <u>AS 3'UT</u>	Probe 179 <u>AS 3,4 loop</u>
10	perivent. hypoth. nu.	+	+
	anter. hypoth. nu.	+	+
	paravent. hypoth. nu.	+½	+½
	nu. horizontal. limb		
	diag. band	+	+
15	ant. amygd. area	++½	++½
	<u>BREGMA -1.80mm</u>		
	reuniens thal.nu.	½+	½+
	rhomboid thal.nu.	½+	½+
	retrochiasmatic area	+	+
20	<u>BREGMA -4.52mm</u>		
	choroid plexus	-	-
	PMCo	+	+
	AHia	+	+
	Basolateral Amygdaloid nu.	++	++
25	dorsal endopiriform nu.	+	+
	hippocampus (all levels)	+	+
	polymorphic dentate gyrus	++	++
	olivary pretectal nu.	++	++
	dorsal lateral genicul. nu.	++	++
30	<u>BREGMA -5.30mm</u>		
	substantia nigra		
	pars reticulata	++	++½
	pars compacta	++	++
	red nucleus parvocellular	-	-
35	retrosplenial cortex	+	+
	occipital cortex	+	+
	nucleus Darkschewitsch	+½	+
	nucleus posterior commis., magnocellular	+	+½
40	<u>BREGMA -7.64mm</u>		
	superior colliculus	+	+
	central grey	-	-
	dorsal grey	+/-	+/-
	median Raphe	+½	+½
45	pontine nuclei	-	-
	Purkinje cells	+/-	+/-

<sup>1</sup> abbreviations as in Paxinos, G. and Watson, C. (1986)  
The Rat Brain in Stereotactic Coordinates, second  
edition. Academic Press.

50

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Table 4 (continued)

2 Antisense probes 191 and 179 were to 3' untranslated  
region and to the 3,4 extracellular loop, respectively.  
5 Control data using sense probes to the same regions  
showed no labeling.

Labeling scale: -, no labeling;  $\frac{1}{2}$ +, very weak, +, weak;  
10 ++, moderate; +++, heavy. Note that the scale is based on  
maximal labeling obtained with GAT-1 probes and should  
not be compared to results for GAT-3.

Table 5. In situ Localization of GAT-3 in the Rat CNS

	<u>Area<sup>1</sup></u>	<u>Labeling</u>
5	<u>telencephalon:</u>	
	cortex	-
	piriform ctx	$\frac{1}{2}+$
	nu. accumbens	
10	core	-
	shell	+
	olf. tubercle	$\frac{1}{2}+$
	med. septal nu.	++
	nu. horiz.limb	
15	diag. band	++
	ventral pallidum	++
	ant. cortical amygdaloid nu.	+
	medial amygdaloid nu.	$+\frac{1}{2}$
20	<u>Diencephalon:</u>	
	paraventricular thalamic nu.	$++\frac{1}{2}$
	reticular thalamic nu.	$+\frac{1}{2}$
	VPM	$+\frac{1}{2}$
	VPL	+
25	zona incerta	$++\frac{1}{2}$
	rhomboid thalamic nu.	$++\frac{1}{2}$
	reuniens thalamic nu.	++
	xiphoid thalamic nu.	+++
	medial geniculate nu.	+
30	arcuate hypoth. nu.	$\frac{1}{2}+$
	ventromedial hypoth.nu.	+
	lateral hypoth. nu.	$+\frac{1}{2}$
	median eminence	$\frac{1}{2}+$
	hippocampus	$\frac{1}{2}+$
35	<u>Mesencephalon:</u>	
	superior colliculus	$++\frac{1}{2}$
	central gray, dorsal	++
	central gray	++
40	substantia nigra	not examined
	interpeduncular nu.	
	caudal	+
	dorsal raphe	+
	cuneiform nu.	+
45	lateral dorsal tegmen. nu.	+++
	dorsal tegmental nu.,	
	pericentral	+++

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Table 5. (continued)

	<u>Area</u> <sup>1</sup>	<u>Labeling</u> <sup>2</sup>
5	<u>Metencephalon:</u>	
	medial vestibular nu.	+++
	lateral superior olive	++
	inferior olive	not examined
	cerebral cortex	-
10	deep cerebellar nuclei	+++

<sup>1</sup> abbreviations as in Paxinos, G. and Watson, C. (1986) The Rat Brain in Stereotactic Coordinates, second edition. Academic Press.

<sup>2</sup> Data are pooled from antisense probes to the 3' untranslated region and to the 3,4 extracellular loop. Control data using sense probes to the same regions showed no labeling.

Labeling scale: -, no labeling;  $\frac{1}{2}$ +, very weak, +, weak; ++, moderate; +++, heavy. Note that the scale is based on maximal labeling obtained with GAT-3 probes and should not be compared to results for GAT-1.

Discussion

The recent cloning of transporters for GABA (21), norepinephrine (55), dopamine (33,65), serotonin (3,23), glycine (68), and taurine (66) has helped to define the structural properties of this class of membrane proteins. In contrast with neurotransmitter receptors, however, it has not been determined for neurotransmitter transporters whether multiple subtypes exist and/or play a role in synaptic transmission. Our identification of two cDNA clones from rat brain encoding novel GABA transporters (designated GAT-2 and GAT-3) provides the first molecular evidence for heterogeneity within the neurotransmitter transporter gene family, and raises the possibility that multiple GABA transporters participate in the regulation of GABAergic neurotransmission.

Both proteins have 12 putative transmembrane domains and can be modeled with a similar topology to the neuronal GABA transporter (GAT-1; (21)), including a large glycosylated extracellular loop between TMs 3 and 4. Analysis of amino acid homologies of the various transporters reveals some unexpected relationships. For example, GAT-2 and GAT-3 exhibit greater amino acid sequence identity to each other (67%) than to GAT-1 (~53%), despite all three transporters displaying nearly identical affinities for GABA. Surprisingly, the sequence closest to GAT-2 and GAT-3 is the dog betaine transporter (79) which, in fact, is as homologous to GAT-2 and GAT-3 as they are to one another. Significantly, the cloned betaine transporter has also been reported to transport GABA (79), although the affinity of GABA at the betaine transporter is nearly 10-fold lower than at GAT-2 and GAT-3. Conversely, the betaine transporter displays at least 10-fold higher affinity for betaine than do GAT-

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2 and GAT-3 (see Table 2). Thus, transporters with as little as 53% amino acid homology can display high affinity for the same substrate (eg. GAT-1 vs. GAT-2 and GAT-3), whereas transporters only slightly more divergent can demonstrate markedly different substrate specificities (eg., GAT-1 vs. glycine, 45% homology; (68)).

Pharmacologically distinct GABA transporters have previously been identified in neuronal and glial cell cultures (15, 36 and 62). Thus, it was of interest to examine the sensitivity of GAT-2 and GAT-3 to a variety of inhibitors and to compare this to published values for endogenous transporters in primary cell cultures, as well as to GAT-1. It is noteworthy that GAT-2 and GAT-3 display greater sensitivity to the glial-selective drug  $\beta$ -alanine than does the previously cloned GAT-1, suggesting similarity to the transporter(s) characterized in glial cell cultures. However, a lack of identity with the pharmacologically defined glial-type transporter is demonstrated by the finding that guvacine, nipecotic acid, Tiagabine, and hydroxynipecotic acid are much less potent inhibitors of GABA uptake at GAT-2 and GAT-3 than at the transporter present in glial cultures (6, 15, 36, 62). Additionally, these compounds are more potent in neuronal cultures (and at the previously cloned GAT-1) than at GAT-2 and GAT-3, which also distinguishes the newly cloned transporters from the neuronal GABA transporter (6, 15, 21, 36 and 62). Lastly, although GAT-2 and GAT-3 display similar sensitivity to a number of the inhibitors examined and show similar affinity for GABA itself, they can be distinguished by L-DABA, which displays higher potency at GAT-2 than at GAT-3. Interestingly, the potency of L-DABA at GAT-2 is similar to that of GAT-1 (Table 2), blurring the distinction



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between the newly cloned transporters and the neuronal-type transporter. This finding may indicate that a spectrum of GABA transport activities underlie the neuronal and glial profiles observed in tissue preparations. Lastly, the three cloned GABA transporters can also be distinguished by their differential dependence on external chloride: GAT-1 is the most chloride dependent, GAT-2 the least, and GAT-3 is intermediate in its sensitivity. The finding that GABA transport by GAT-2 and GAT-3 is not completely eliminated in chloride-free medium suggests that their mechanism of transport is fundamentally different from that of GAT-1.

It is somewhat surprising that the pharmacological profiles of GAT-2 and GAT-3 differ from those of previously characterized transporters in neuronal and glial cultures. One possible explanation is that the unique pharmacology of GAT-2 and GAT-3 reflects species differences, as the cloned transporters were obtained from a rat cDNA library, while mouse tissue was employed in many of the earlier studies (15, 36 and 62). This hypothesis gains validity from the finding that certain GABA uptake blockers are potent anticonvulsants in rats, but are ineffective in mice (82), although differences in drug metabolism or distribution have not been ruled out. A second possibility is that since neuronal and glial cultures are prepared from fetal or newborn animals, the discrepant results may reflect developmental changes in GABA transporters or peculiarities of glia and neurons when maintained in cell culture. Alternatively, the two newly cloned transporters may in fact represent members of a novel class of transporters that have not been previously identified, perhaps due to their low abundance in cultured cells. This would suggest that further GABA transporters with pharmacological profiles consistent

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with those seen in neuronal and glial cultures remain to be cloned. Lastly, it should be pointed out that the pharmacological profiles of cloned transporters for serotonin (3,23), dopamine (33,65), and norepinephrine (55), as well as GAT-1 are similar to those observed in brain homogenates, thus arguing that the unique properties of GAT-2 and GAT-3 are not the result of the heterologous expression system.

Despite the generally similar pharmacology of GAT-2 and GAT-3, their patterns of distribution are distinct. All three high-affinity GABA transporters are present in brain and retina, while only GAT-2 was detected in peripheral tissues. This finding is consistent with recent studies suggesting a role for GABA in liver (52), kidney (1,19) and other peripheral tissues (for review, ref. 14). Further distribution studies of GAT-2 and GAT-3 by *in situ* localization of transporter mRNAs in conjunction with immunocytochemistry will help to define the roles of these transporters in GABAergic transmission.

In conclusion, we now report the identification in mammalian brain of two novel high-affinity GABA transporters with unique pharmacological properties. These studies indicate previously unsuspected complexity in the regulation of GABAergic transmission, and provide the opportunity for the development of selective therapeutic agents to treat neurological and psychiatric disorders.

#### Cloning of Human High-Affinity GABA Transporters:

The use of human gene products in the process of drug development offers significant advantages over those of other species, which may not exhibit the same

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pharmacologic profiles. To facilitate this human-target based approach to drug design in the area of inhibitory amino acid transporters, we used the nucleotide sequences of the rat GAT-2 and GAT-3 cDNAs to clone the human homologues of each gene.

To obtain a cDNA clone encoding the human GAT-2 GABA transporter (hGAT-2) we used PCR primers based on the rat GAT-2 sequence to detect the presence of hGAT-2 in human cDNA libraries. PCR was carried out at a reduced annealing temperature to allow mismatches between rat and human sequences (see Experimental Procedures); amplified hGAT-2 sequences were detected by hybridization at low stringency with radiolabeled (randomly primed) rat GAT-2 cDNA. A human heart cDNA library (Stratagene) was identified and screened at low stringency with the same probe, resulting in isolation of a partial cDNA clone (hHE7a) containing the C-terminal portion of the coding region of hGAT-2. Using human sequence derived from this clone, a partial cDNA clone (hS3a) was isolated from a human striatum cDNA library (Stratagene) that provided additional sequence in the coding region. The hGAT-2 nucleotide sequence from these two clones and the deduced amino acid sequence based on translation of a long open reading frame is shown in Figure 10A. The sequence includes 738 base pairs of coding region (246 amino acids) and 313 base pairs of 3' untranslated region. Comparison with the rat GAT-2 amino acid sequence reveals 90% identity over the region encoded by the clones, which includes predicted transmembrane domains 8-12 and the carboxy terminus of hGAT-2.

To obtain the nucleotide sequence of the human GAT-3 GABA transporter (hGAT-3), degenerate PCR primers were used to amplify transporter sequences from human cDNA libraries.

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Amplified hGAT-3 sequences were detected in the library by hybridization at low stringency with radiolabeled oligonucleotides representing the region of the rat GAT-3 cDNA that encodes a portion of the second extracellular loop. The human fetal brain library (Stratagene) identified by this approach was screened at high-stringency with the same probes; positive plaques were purified by successive screening at low stringency. Two cDNA clones were isolated (hFB16a, hFB20a) which together comprise nearly the entire coding region of hGAT-3; the sequence of the remaining 7 base pairs was supplied by a genomic clone (hp28a) isolated from a human placental library. A vector comprising the complete coding sequence of hGAT-3 was constructed using appropriate fragments of these three clones, and is designated pcEXV-hGAT-3. The complete nucleotide sequence and predicted amino acid sequence of hGAT-3 are shown in Figure 10B. In addition to 1896 base pairs of coding region, the sequence includes 5' and 3' untranslated sequence (34 and 61 base pairs, respectively). Translation of a long open reading frame predicts a protein of 632 amino acids that is 95% identical to the rat GAT-3 and contains 12 putative transmembrane domains. Methods similar to methods used to clone the human homologues of the mammalian GABA transporters can similarly be used to clone the human homologues of the mammalian taurine transporter.

The cloning and expression of the human GAT-2 and GAT-3 will allow comparison of pharmacological profiles with those of rat GABA transporters, and also provide a means for understanding and predicting the mechanism of action of GABA uptake inhibitors as human therapeutics. Recently, several additional transporters have been cloned which exhibit significant sequence homology with

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previously cloned neurotransmitter transporters. cDNA and genomic clones representing the mouse homologues of GAT-1 were recently reported (39). In addition, a glycine transporter cDNA that is similar but not identical to that cloned by Smith et al. (68) was cloned from both rat (22) and mouse (39). A high-affinity L-proline transporter was reported by Freneau et al. (18), supporting a role for L-proline in excitatory neurotransmission. A rat cDNA identified as a choline transporter was reported by Mayser et al. (50). A taurine transporter cDNA was recently cloned from dog kidney cells (74) which is 90% identical to the rat taurine transporter amino acid sequence reported by Smith et al. (66). A cDNA encoding a mouse GABA transporter was recently cloned by Lopez-Corcuera et al. (45); the transporter encoded by this cDNA is 88% identical to the dog betaine transporter (79), and may represent the mouse homologue of that gene. Finally, a  $\beta$ -alanine-sensitive GABA transporter from rat brain has been cloned (10) that exhibits 100% amino acid identity with the rat GAT-3 sequence reported by Borden et al. (4).

## 2. Taurine

### Results and Discussion

#### 25 Cloning of Mammalian Taurine Transporter:

We screened a rat brain cDNA library at low stringency with probes encoding the rat brain GABA transporter GAT-1 (21) in order to identify additional inhibitory amino acid transporter genes. Several clones were isolated which hybridized at low but not at high stringency with the GABA transporter probes. Characterization of the clones by DNA sequence analysis revealed that they represented a novel transporter sequence related to GAT-1. None of the clones contained the complete coding region of the putative transporter, and thus the library

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was rescreened at high stringency using oligonucleotides designed from the new sequence. A 2.5 kb cDNA clone (designated rB16a) was isolated which contained an open reading frame of 1863 base pairs encoding a protein of 621 amino acids (Figure 1C). Comparison of this sequence with the rat GABA transporter cDNA revealed 58% nucleotide identity within the coding region. Comparison with sequences in Genbank and EMBL data bases demonstrated that the sequence was novel and that the most closely related sequence was the rat GABA transporter (21) followed by the human norepinephrine transporter (55). Subsequent comparisons to recently cloned transporters indicate that the most homologous sequences are two novel GABA transporters designated GAT-2 and GAT-3 (4) and the betaine transporter (79), which exhibit 62-64% nucleotide identity with rB16a.

The amino acid sequence deduced from the nucleotide sequence of rB16a is shown in Figure 1E with a membrane topology similar to that proposed for the rat GABA transporter (21) and other cloned neurotransmitter transporters (3, 23, 33, 55 and 65). The translation product of rB16a is predicted to have a relative molecular mass of ~70,000 Daltons. Hydropathy analysis indicates the presence of 12 hydrophobic domains which may represent membrane spanning segments. Three potential sites for Asn-linked glycosylation are found in the extracellular loop between the third and fourth transmembrane domains. Alignment of the deduced amino acid sequence of rB16a with the rat GABA transporter (GAT-1; (21)) and the dog betaine transporter (79) revealed 50% and 58% amino acid identities, respectively (Figure 6). Comparison of rB16a with the glycine transporter (Figure 6; (68)) and the human norepinephrine transporter (55) also showed significant amino acid

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homology (41-45%), similar to that between GAT-1 and the norepinephrine transporter (46%). As predicted from nucleotide comparisons, the strongest amino acid homology (~61%) is with the GABA transporters GAT-2 and GAT-3 recently cloned from rat brain (4). In contrast, the sodium/glucose cotransporter (22), which shows a low degree of homology with cloned neurotransmitter transporters, displays only 21% amino acid identity with rB16a. These data suggested that the new sequence might encode an inhibitory amino acid transporter expressed in the brain. To explore this possibility, rB16a was placed in a mammalian expression vector, transfected into COS cells, and screened for transport of a variety of radiolabeled neurotransmitters and amino acids.

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Pharmacological Characterization of Mammalian Taurine Transporter:

COS cells transiently transfected with rB16a (COS/rB16a) accumulated approximately 6-fold more [ $^3\text{H}$ ]taurine than control, non-transfected cells (Figure 7). Specific uptake represented greater than 95% of total uptake in transfected cells. In contrast, the uptake of [ $^3\text{H}$ ]glutamate, [ $^3\text{H}$ ]glycine, [ $^3\text{H}$ ]5-HT, [ $^3\text{H}$ ]dopamine, and [ $^3\text{H}$ ]GABA was unaltered. Uptake of [ $^3\text{H}$ ]taurine was not observed following mock transfection, indicating that the enhanced uptake was not the result of non-specific perturbation of the membrane. The transport of [ $^3\text{H}$ ]taurine by COS/rB16a was decreased >95% when  $\text{Na}^+$  was replaced by  $\text{Li}^+$ , or when  $\text{Cl}^-$  was replaced by acetate (Figure 7). In the absence of sodium or chloride, taurine transport in COS/rB21a decreased to levels below that of non-transfected controls, demonstrating that endogenous taurine transporter activity present in COS cells is also dependent on these ions. A similar ion dependence has been observed for taurine transport in

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vivo (27), as well as for the activity of other cloned neurotransmitter transporters such as those for GABA (21), glycine (68), and norepinephrine (55).

5 To determine the affinity of taurine for the cloned transporter, COS/rB16a was incubated with various concentrations of [<sup>3</sup>H]taurine and the specific accumulation of radioactivity was determined. Accumulation of [<sup>3</sup>H]taurine was dose-dependent and  
10 reached saturation at higher concentrations (Figure 8). Non-linear regression analysis of the data yielded the following values:  $K_M = 43 \pm 6 \mu M$ , and  $V_{MAX} = 0.96 \pm 0.27$  nmoles/mg protein (mean  $\pm$  SEM, n=4 experiments). The affinity of the cloned transporter for taurine is similar  
15 to that of high-affinity taurine transporters in both the central nervous system (42,80) and peripheral tissues (37) which exhibit  $K_M$  values from 10 to 60  $\mu M$ . Taken together, these data indicate that rB16a encodes a saturable, high-affinity, sodium- and chloride-dependent  
20 taurine transporter.

To determine the pharmacological specificity of the cloned transporter, various agents were examined for their ability to inhibit the transport of [<sup>3</sup>H]taurine by  
25 COS/rB16a (Table 6). As the endogenous taurine transporter in COS cells accounted for, on average, 16% of the total transport activity observed in transfected cells, we were concerned that this could influence results. Accordingly, we also examined the sensitivity  
30 of the endogenous taurine transporter present in non-transfected cells. As shown in Table 6, the pharmacologic properties of the cloned taurine transporter closely matched those of the endogenous transporter and thus did not lead to erroneous results.

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The most potent inhibitors were taurine and hypotaurine, each of which inhibited specific [ $^3\text{H}$ ]taurine uptake approximately 30-40% at 10 $\mu\text{M}$ , 90% at 100 $\mu\text{M}$ , and 100% at 1mM.  $\beta$ -alanine was slightly less potent, inhibiting specific uptake 15%, 51%, and 96% at 10 $\mu\text{M}$ , 100 $\mu\text{M}$ , and 1mM, respectively; the high potency of  $\beta$ -alanine as an inhibitor of taurine uptake is consistent with the finding that COS/rB16a showed a 6-fold increase in the specific uptake of [ $^3\text{H}$ ] $\beta$ -alanine (data not shown), essentially identical to the fold-increase observed with [ $^3\text{H}$ ]taurine. The taurine analogue GES was also quite potent, inhibiting specific uptake of [ $^3\text{H}$ ]taurine 11%, 45% and 92% at 10 $\mu\text{M}$ , 100 $\mu\text{M}$  and 1mM, respectively. APSA and GABA both inhibited uptake approximately 10% and 40% at 100 $\mu\text{M}$  and 1mM, respectively. The observations that GABA is a poor inhibitor of taurine uptake, and that transfection with rB16a did not result in enhanced uptake of [ $^3\text{H}$ ]GABA (see above), are consistent with the previous report (38) that GABA is a weak non-competitive inhibitor of taurine uptake. Less than 10% inhibition of [ $^3\text{H}$ ]taurine uptake was observed for the following compounds (each tested at 1mM): the structural analogues AEPA and MEA as well as the sulfur-containing amino acids cysteine and methionine (Table 6), and (data not shown) norepinephrine, dopamine, glutamate, glycine, serine, betaine, L-methionine, and  $\alpha$ -methylaminoisobutyric acid (a substrate for amino acid transporter designated system A; (21)). Taken together, these results indicate that the taurine transporter encoded by rB16a is similar to the endogenous taurine transporter in COS cells (Table 6), as well as the endogenous taurine transporter(s) present in neural tissue (25), (see also ref. 27 and references therein).

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It is of interest that sensitivity to  $\beta$ -alanine is shared by the two high-affinity GABA transporters recently cloned from rat brain (GAT-2 and GAT-3 (4)), which are even more closely related to the taurine transporter (62% amino acid identity) than to the neuronal-type GABA transporter GAT-1 (51%).  $\beta$ -alanine has been shown to activate an inward chloride current in spinal neurons (9,49) and is released in a calcium-dependent manner from several brain areas (31,58), suggesting a role as an inhibitory neurotransmitter in the CNS. The similar sensitivities of the newly cloned GABA transporters (4) and the taurine transporter to  $\beta$ -alanine, combined with their sequence homologies, suggest that they represent a subfamily of inhibitory neurotransmitter transporters. Despite these similarities, these transporters unexpectedly exhibit widely divergent affinities for GABA: GAT-2 and GAT-3 show the highest affinity ( $K_m=10\mu M$  (4)), while the affinity of the taurine transporter is extremely low ( $\sim 1mM$ , Table 6). Interestingly, the dog betaine transporter (79), which displays a similar degree of homology to the members of this subfamily (ca. 60%), exhibits an intermediate affinity for GABA ( $\sim 100\mu M$ ). The finding that four structurally related transporters display overlapping substrate specificities for the neuroactive amino acids GABA and  $\beta$ -alanine suggests that multiple transporters may regulate the synaptic levels of these substances. This crossreactivity underscores the importance of understanding the action of therapeutic agents at both GABA and taurine transporters.

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Table 6. Pharmacological Specificity of [<sup>3</sup>H]taurine Uptake.

	<u>Inhibitor<sup>a</sup></u>	<u>Concentration</u>	<u>% Inhibition</u>	
			<u>control</u>	<u>rB16a</u>
5	AEPA	1mM	0±0 (4)	3±3 (5)
	AMSA	1mM	1±1 (4)	7±3 (4)
10	APSA	100μM	7±3 (4)	8±4 (4)
		1mM	45±3 (5)	36±4 (5)
	β-alanine	10μM	9±2 (6)	15±6 (6)
		100μM	63±3 (6)	51±4 (10)
15		1mM	97±1 (4)	96±1 (8)
	CSA	1mM	2±1 (4)	7±5 (3)
	cysteine	1mM	4±3 (3)	2±2 (3)
20	GABA	10μM	1±1 (4)	9±6 (4)
		100μM	9±4 (6)	10±4 (10)
		1mM	49±2 (5)	44±6 (8)
25	GES	10μM	6±3 (4)	11±4 (4)
		100μM	47±3 (5)	45±5 (5)
		1mM	89±1 (5)	92±1 (6)
	hypotaurine	10μM	41±3 (7)	26±7 (7)
30		100μM	91±1 (4)	84±3 (4)
		1mM	99±1 (4)	100±1 (4)
	MEA	1mM	1±0 (3)	3±3 (4)
35	methionine	1mM	1±1 (3)	1±1 (3)

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Table 6 (continued)

5	taurine	10 $\mu$ M	38 $\pm$ 5 (7)	29 $\pm$ 8 (5)
		100 $\mu$ M	89 $\pm$ 2 (4)	83 $\pm$ 2 (5)
		1mM	100 <sup>b</sup>	100 <sup>b</sup>

10 a Non-transfected COS-7 cells (control), or COS-7 cells transfected with rB16a were incubated for 10 minutes (37°C) with 50nM [<sup>3</sup>H]taurine and the indicated compounds. Data show percent displacement of specific [<sup>3</sup>H]taurine uptake (mean $\pm$ SEM; values in parentheses indicate number of experiments).

15 <sup>b</sup> Non-specific uptake defined with 1mM taurine.

20 Abbreviations: AEPA, 2-aminoethylphosphonic acid; AMSA, aminomethanesulfonic acid; APSA, 3-amino-1-propanesulfonic acid; CSA, cysteinesulfinic acid; GABA, gamma-aminobutyric acid; GES, guanidinoethanesulfonic acid; MEA, 2-mercaptoethylamine.

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Tissue Localization Studies of Mammalian Taurine  
Transporter:

To define the tissue distribution patterns of the taurine transporter, polymerase chain reaction (PCR) was used to detect the rB16a sequence in cDNA representing mRNA from seven different rat tissues. As a control, the distribution of the constitutively expressed protein cyclophilin was also examined. Radiolabeled oligonucleotides specific for rB16a were used to detect PCR products by hybridization. As shown in Figure 9A, the taurine transporter was detectable in all tissues examined, including brain, retina, liver, kidney, heart, spleen, and pancreas, after 30 cycles of PCR. Cyclophilin was amplified to a similar extent from all the tissues (data not shown), demonstrating that adequate cDNA was present in each sample.

To evaluate both the abundance and the size of the mRNA encoding the taurine transporter, Northern blot analysis was carried out on poly A+ RNA isolated from the same rat tissues used for PCR analysis, with the addition of lung. As shown in Figure 9B, a single -6.2 kb transcript which hybridized with the taurine transporter cDNA probe was detected in brain, kidney, heart, spleen, and lung after an overnight exposure of the autoradiogram. After a 3-day exposure, bands of the same size were also visible in liver and pancreas (data not shown). Rehybridization of the blot with the cDNA encoding cyclophilin (12) confirmed that roughly equal amounts of RNA were present in each sample except that of retina, which was significantly degraded (data not shown). Thus, taurine transporter mRNA levels were highest in brain and lung, intermediate in kidney, heart, and spleen, and lowest in liver and pancreas. The abundance and pattern of distribution of the taurine transporter mRNA by Northern

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blot are consistent with data obtained using PCR (Figure 9); further, the same size transcript is present in all tissues evaluated. These findings suggest that a single taurine transporter functions in both the brain and peripheral tissues; however, we can not exclude the existence of additional taurine transporters.

Taurine is abundant in the central nervous system and is involved in a variety of neural activities. Unlike classical neurotransmitters, the effects of taurine are mediated both intra- and extracellularly. A major regulator of taurine levels, both within cells and in the synaptic cleft, is the transport of taurine across the plasma membrane. Our cloning of a high-affinity taurine transporter represents a critical step in defining the role of taurine in both neural and non-neural tissues, and in the development of therapeutic agents that alter taurine and GABA neurotransmission. In addition, the identification of a new member of the set of inhibitory amino acid transporters will aid in elucidating the molecular structure-function relationships within the transporter family.

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REFERENCES

1. Amenta, F., Cavallotti, C., Iacopono, L., and Erdo, S.L. 36, 390-395.  
5
2. Andrade, R., Malenka, R.C., and Nicoll, R.A. (1988) Science 234, 1261-1265.
3. Blakely, R. D., Berson, H. E., Fremeau, Jr., R. T., Caron, M. G., Peek, M. M., Prince, H. K., and Bradley, C. C. (1991). Nature 354, 66-70.  
10
4. Borden, L.A., K.E. Smith, P.R. Hartig, T.A. Branchek, and R.L. Weinshank (1992) J. Biol. Chem. In press.  
15
5. Bowery, N.G., G.P. Jones, and M.J. Neal (1976) Nature (London) 264, 281-284.
6. Braestrup, C., Nielsen, E.B., Sonnewald, U., Knutsen, L.J.S., Andersen, K.E., Jansen, J.A., Frederiksen, K., Andersen, P.H., Mortensen, A., and Suzdak, P.D. (1990) J. Neurochemistry 54, 639-647.  
20
7. Capecchi M.R., Science 244, 1288-1292 (1989)  
25
8. Chadwick, D., Richens, A., Duncan, J., Dam, M., Gram, L., Morrow, J., Mengel, H., Shu, V., McKelvy, J.F., and Pierce, M.W. (1991) Epilepsia 32 (supplement 3), 20.  
30
9. Choquet, D. and Korn, H. Does  $\beta$ -alanine activate more than chloride channel associated receptor? Neurosci. Letters 84:329-340 (1988).  
35

-114-

10. Clark, J.A., A.Y. Deutch, P.Z. Gallipoli, and S.G. Amara (1992) *Neuron* 9, 337-348.
11. Cohen, J. S., *Trends in Pharm. Sci.* 10, 435 (1989).
12. Danielson, P.E., Forss-Petter, S., Brow, M.A., Calavetta, L., Douglass, J., Milner, R.J., and Sutcliffe, J.G. (1988). *DNA* 7, 261-267.
13. Dichter, M.A. (1980) *Brain Res.* 190, 111-121.
14. Erdo, S. L. and Wolff, J.R. (1990) *J. Neurochem.* 54, 363-372.
15. Falch, E., Larsson, O.M., Schousboe, and Krogsgaard-Larsen, P. (1990). *Drug Devel. Res.* 21, 169-188.
17. Feinberg, A. P.; and Bogelstein, B. (1988). A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132, 6-13.
18. Freneau, R.T., Jr., M.G. Caron, and R.D. Blakely (1992) *Neuron* 8, 915-926.
19. Goodyer, P.R., Rozen, R., and Sriver, C.R. (1985) *Biochem. Biophys. Acta* 818, 45-54.
20. Guastella, J., N. Brecha, C. Wiegmann, H.A. Lester, and N. Davidson (1992) *Proc. Natl. Acad. Sci. USA* 89, 7189-7193.



-115-

21. Guastella, J., N. Nelson, H. Nelson, L. Czyzyk, S. Keynan, M. C. Miedel, N. Davidson, H. A. Lester, and B. I. Kanner (1990) *Science* 249:1303-6.
- 5 22. Hediger, M.A., Turk, E., and Wright, E.M. Homology of the human intestinal Na<sup>+</sup>/glucose and *Escherichia coli* Na<sup>+</sup>/proline cotransporters. *Proc. Natl. Acad. Sci. USA* 86:5748-5752.
- 10 23. Hoffman, B. J., Mezey, E., and Brownstein, M. J. Cloning of a serotonin transporter affected by antidepressants. *Science* 254: 579-580 (1991).
- 15 24. Hogan B. et al., *Manipulating the Mouse Embryo, A Laboratory Manual*, Cold Spring Harbor Laboratory (1986).
- 20 25. Hruska, R.E., Huxtable, R.J., and Yamumura, H.I. High-affinity, temperature-sensitive, and sodium-dependent transport of taurine in rat brain. in *Taurine and Neurological Disorders*, ed. A. Barbeau and R.J. Huxtable. (Raven Press, NY, 1978).
- 25 26. Huxtable, R.J. Review: Taurine interactions with ionic conductances in excitable membranes. *Prog. Clin. Biol. Res.* 351:157-161 (1990).
- 30 27. Huxtable, R.J. Taurine in the central nervous system and the mammalian actions of taurine. *Prog. Neurobiol.* 32:471-533 (1989).
28. Iversen, L.L. and Bloom, F.E. (1972) *Brain Res.* 41, 131-143.

-116-

29. Kanner, B. I. and Schuldiner, S. (1987) *CRC Crit, rev. Biochem.* 22, 1-38.
- 5 30. Kanner, B. I. and A. Bendahan (1990) *Proc. Natl. Acad. Sci. USA* 87, 2550-2554.
- 10 31. Kihara, M., Misu, Y., and Kubo, T. Release by electrical stimulation of endogenous glutamate,  $\gamma$ -aminobutyric acid, and other amino acids from slices of the rat medulla oblongata. *J. Neurochem.* 52:261-267 (1989).
- 15 32. Kilberg, M.S. Amino acid transport in isolated rat hepatocytes. *J. Memb. Biol.* 69:1-12 (1982).
33. Kilty, J. E., Lorang D., and Amara, S. G. (1991). *Science* 254, 578-579.
- 20 34. Kontro, P., Korpi, E.R., and Oja, S.S. Taurine interacts with GABA<sub>A</sub> and GABA<sub>B</sub> receptors in the brain. *Prog. Clin. Biol. Res.* 351:83-94 (1990).
- 25 35. Krnjevic, K. (1991) in *GABA Mechanisms in Epilepsy*, ed. G. Tunnickliff and B.U. Raess, pp 47-87, Wiley-Liss, NY.
- 30 36. Krogsgaard-Larsen, P., Falch, E., Larsson, O.M., and Schousboe, A. (1987) *Epilepsy Res.* 1, 77-93.
- 37 Lambert, I.H. and Hoffman, E.K. Taurine transport and cell volume regulation in a mammalian cell. *Prog. Clin. Biol. Res.* 351:267-276 (1990).
- 35 38. Larsson, O.M, Griffiths, R., Allen, I.C., and Schousboe, A. Mutual inhibition kinetic analysis of

-117-

- 5         $\gamma$ -aminobutyric acid, taurine, and  $\beta$ -alanine high-affinity transport into neurons and astrocytes: Evidence for similarity between the taurine and  $\beta$ -alanine carriers in both cell types. *J. Neurochem.* 47:426-432 (1986).
- 10        39. Liu, Q.-R., H. Nelson, S. Mandiyan, B. Lopez-Corcuera, and N. Nelson (1992a) *FEBS Letters* 305,110-114.
- 15        40. Liu, Q.-R., S. Mandiyan, H. Nelson, and N. Nelson (1992) *Proc. Natl. Acad. Sci. USA* 89,6639-6643.
- 20        41. Lombardini, J.B. (1988) Effects of taurine and mitochondrial metabolic inhibitors on ATP-dependent  $\text{Ca}^{2+}$  uptake in synaptosomal and mitochondrial subcellular fractions of rat retina, *J. Neurochemistry* 51, 200-205.
- 25        42. Lombardini, J.B. High-affinity transport of taurine in the mammalian central nervous system, in *Taurine and Neurological Disorders*, (A. Barbeau and R. J. Huxtable, eds.). Raven Press, New York, 119-135 (1978).
- 30        43. Lombardini, J.B. and Kiebowitz, S.M. (1990) Inhibitory and stimulatory effects of structural and conformational analogues of taurine on ATP-dependent calcium ion uptake in the rat retina: Deductions concerning the conformation of taurine. In *Progress in Clinical and Biological Research* 351, 197-206.
- 35        44. Lopata, M. A., Cleveland, D. W., and Sollner-Webb, B. (1984). *Nucl. Acids Res.* 12, 5707-5717.

-118-

45. Lopez-Corcuera, B., Q.-R. Liu, S. Mandiyan, H. Nelson, and N. Nelson (1992) *J. Biol. Chem.* 267,17491-17493.
- 5      46. Low, M.J., Lechan, R.M., and Hammer, R.E. (1986) *Science* 231, 1002-1004.
- 10      47. Maniatis, T., Fritsch, E.F. Fritsch and Sambrook, J., *Molecular Cloning*, Cold Spring Harbor Laboratory, 1982.
- 15      48. Maniatis , T., Fritsch, E.F. and Sambrook, J., *Molecular Cloning*, Cold Spring Harbor Laboratory, pp 197-198, 1982.
- 20      49. Mathers, D.A., Grewal, A., and Wang, Y.  $\beta$ -alanine induced ion channels in the membrane of cultured spinal cord neurons. *Neurosci. Letters* 108:127-131 (1990).
- 25      50. Mayser, W., P. Schloss, and H. Betz (1992) *FEBS Letters* 305, 31-36.
51. Miller, J., and Germain, R.N. (1986). *J. Exp. Med.* 164, 1478-1489.
52. Minuk, G.Y., Vergalla, J., Ferenci, P., and Jones, E.A. (1984) *Hepatology* 4, 180-185.
- 30      53. Neal, M. J. and N. G. Bowery (1977) *Brain Res.* 86, 243-257.
- 35      54. Oberdick, J., Smeyne, R.J., Mann, J.R., Jackson, S. and Morgan, J.I. (1990) *Science* 248, 223-226.

-119-

55. Pacholczyk, T., Blakely, R.D., and Amara, S.G. Expression cloning of a cocaine- and antidepressant-sensitive human noradrenaline transporter. *Nature* 350:350-354 (1991).
- 5
56. Quinn, M.R. Taurine allosterically modulates binding sites of the GABA<sub>A</sub> receptor. *Prog. Clin. Biol. Res.* 351:121-127 (1990).
- 10
57. Rogawski, M.A. and Porter, R.J. (1990) *Pharmacological Reviews* 42, 224-286.
58. Sandberg, M. and Jacobson, I.  $\beta$ -alanine, a possible neurotransmitter in the visual system? *J. Neurochem.* 37:1353-1356 (1981).
- 15
59. Sanger, S. (1977). *Proc. Natl. Acad. Sci. USA* 74, 5463-5467.
- 20
60. Sarver, N. et al., *Science* 247, 1222 (1990)).
61. Schon, F. and J. S. Kelly (1975) *Brain Res.* 41, 131-143.
- 25
62. Schousboe, A., Larsson, O.M., and Krogsgaard-Larsen, P. (1991) in *GABA Mechanisms in Epilepsy*, ed. G. Tunnichliff and B.U. Raess, pp 165-187, Wiley-Liss, NY.
- 30
63. Sedman, A.J., Gilmet, G.P., Sayed, A.J., and Posvar, E.L. (1990) *Drug Development Research* 21, 235-242.
64. Shain, W., and Martin, D.L. Review: Uptake and release of taurine: an overview. *Prog. Clin. Biol. Res.* 351:243-252 (1990).
- 35

-120-

65. Shimada, S., Kitayama, S., Lin, C.-L., Patel, A.,  
Nanthakumaar, E., Gregor, P. Kuhar, M. and Uhl, G.  
(1991). *Science* 254, 576-578.
- 5 66. Smith, K.E., L.A. Borden, C.-H.D. Wang, P.R. Hartig,  
T.A. Branchek, and R.L. Weinshank (1992a) *Mol.*  
*Pharm.* In press.
- 10 67. Smith, K. E., Borden, L. A., Branchek, T., Hartig,  
P. R., and Weinshank, R. L. DNA encoding a glycine  
transporter and uses thereof. Pat. Pending.
- 15 68. Smith, K.E., L.A. Borden, P.R. Hartig, T.A.  
Branchek, and R.L. Weinshank (1992) *Neuron* 8, 927-  
935.
- 20 69. Smullin, D.H., Schamber, C.D., Skilling, S.R., and  
Larson, A. A. (1990) A possible role for taurine in  
analgesia. In *Progress in Clinical and Biological*  
*Research* 351, 129-132.
70. Sturman, J.A. Review: Taurine deficiency. *Prog.*  
*Clin. Biol. Res.* 351:385-395 (1990).
- 25 71. Tallman, J.F. and Hutchison, A. (1990) Molecular  
biological insights into GABA and benzodiazepine  
receptor structure. in *Progress in Clinical and*  
*Biological Research* 361, 131-144.
- 30 72. Taylor, C.P., Vartanian, M.G., Schwarz, R.D., Rock,  
D.M., Callahan, M.J., and Davis, M.D. (1990) *Drug*  
*Development Research* 21, 195-215.

-121-

73. Twyman, R.E. and Macdonald, R. L. (1991) in *GABA Mechanisms in Epilepsy*, editors G. Tunnichliff and B.U. Raess, pp 89-104, Wiley-Liss, NY.
- 5 74. Uchida, S., H. M. Kwon, A. Yamauchi, A.S. Preston, F. Marumo, and J. Handler (1992) *Proc. Natl. Acad. Sci. USA* 89, 8230-8234.
- 10 75. Van Gelder, N.M. Neuronal discharge hypersynchrony and the intracranial water balance in relation to glutamic acid and taurine redistribution: Migraine and epilepsy. *Prog. Clin. Biol. Res.* 351:1-20 (1990).
- 15 76. Weintraub, H.M., *Scientific American*, January (1990) p. 40.
- 20 77. Williams, M. (1990) in *Progress in Clinical and Biological Research* 361, ed. B.S. Meldrum and M. Williams, pp 131-144, Wiley-Liss, NY.
- 25 78. Wu, J.-Y., Liao, C., Lin, C.J., Lee, Y.H., Ho, J.-Y., and Tsai, W.H. (1990) Taurine receptor in mammalian brain. in *Progress in Clinical and Biological Research* 351, 147-156.
- 30 79. Yamauchi, A., S. Uchida, H.M. Kwon, A.S. Preston, R.B. Robey, A. Garcia-Perez, M.B. Burg, and J.S. Handler (1992) *J. Biol. Chem.* 267, 649-652.
- 35 80. Yorek, M.A. and Spector, A.A. Taurine transport and metabolism in human retinoblastoma cells, in *Taurine: Biological actions and clinical perspectives*. Alan R. Liss, Inc. 361-370 (1985).

-122-

81. Yunger, L.M., Fowler, P.J., Zarevics, P., and Setler, P.E. (1984) J. Pharmacol. Experimental Therapeutics 228, 109-115
- 5 82. Zimmer, A. and Gruss, P., Nature 338, 150-153 (1989).
83. Hammer, R.E. et al., Science 231: 1002-1004 (1986).
- 10 84. Morgan, J.I., Science 248: 223-226 (1986).
85. Branchek, T., Adham, A., Macchi, M., Kao, H.T. and Hartig, P. R., Molecular Pharmacology 36: 604-609 (1990).
- 15 86. Kanner, B.I., Biochemistry 17: 1207-1211 (1978).
87. Mabjeesh, N.J., Frese, M., Rauen, T., Jeserich, G. and Kanner B.I., Federation of European Biochemical Societies 299: 99-102 (1992).
- 20 88. Rudnick, G., Journal of Biological Sciences 252: 2170-2174 (1977).



## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Smith, E. Kelli  
Borden, A. Laurence  
Hartig, R. Paul  
Weinshank, L. Richard
- (ii) TITLE OF INVENTION: DNA ENCODING TAURINE AND GABA TRANSPORTERS AND  
USES THEREOF
- (iii) NUMBER OF SEQUENCES: 10
- (iv) CORRESPONDENCE ADDRESS:  
(A) ADDRESSEE: Cooper & Dunham  
(B) STREET: 30 Rockefeller Plaza  
(C) CITY: New York  
(D) STATE: New York  
(E) COUNTRY: USA  
(F) ZIP: 10112
- (v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.24
- (vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:  
(A) NAME: White, John  
(B) REGISTRATION NUMBER: 28,678  
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## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 2028 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: both  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: N
- (iv) ANTI-SENSE: N
- (v) FRAGMENT TYPE: N-terminal
- (vii) IMMEDIATE SOURCE:  
(A) LIBRARY: rat brain

(B) CLONE: rB14b

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 126..1932

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGCAGCGAAC ACAAGCGCAT CCGGTAGAAC GGAAAGAACA GGAATTGCAG AGTGA	60
CTTCA AGTCTCCATA CGATTACTA CCCGGGTGAC GGCAGTGA	120
CTGACGAGTA GCGGCTGCAG GTGGG ATG GAT AAC AGG GTC TCG GGA	167
ACG ACC AGT AAT GGA GAG ACA Met Asp Asn Arg Val Ser Gly Thr Thr Ser Asn Gly Glu Thr	
1 5 10	
AAG CCA GTG TGT CCA GTC ATG GAG AAG GTG GAG GAA GAC GGT ACC TTG	215
Lys Pro Val Cys Pro Val Met Glu Lys Val Glu Glu Asp Gly Thr Leu	
15 20 25 30	
GAA CGG GAG CAA TGG ACC AAC AAG ATG GAG TTC GTA CTG TCA GTG GCG	263
Glu Arg Glu Gln Trp Thr Asn Lys Met Glu Phe Val Leu Ser Val Ala	
35 40 45	
GGA GAG ATC ATT GGC TTA GGC AAC GTC TGG AGG TTT CCC TAT CTC TGC	311
Gly Glu Ile Ile Gly Leu Gly Asn Val Trp Arg Phe Pro Tyr Leu Cys	
50 55 60	
TAC AAG AAC GGG GGA GGT GCC TTC TTT ATT CCC TAC CTC ATC TTC CTA	359
Tyr Lys Asn Gly Gly Gly Ala Phe Phe Ile Pro Tyr Leu Ile Phe Leu	
65 70 75	
TTT ACC TGT GGC ATT CCT GTC TTC TTC CTG GAG ACA GCG CTT GGC CAG	407
Phe Thr Cys Gly Ile Pro Val Phe Phe Leu Glu Thr Ala Leu Gly Gln	
80 85 90	
TAC ACC AAC CAG GGA GGC ATC ACA GCC TGG AGG AAA ATC TGT CCC ATC	455
Tyr Thr Asn Gln Gly Gly Ile Thr Ala Trp Arg Lys Ile Cys Pro Ile	
95 100 105 110	
TTC GAG GGC ATC GGC TAT GCC TCA CAG ATG ATC GTC AGC CTT CTC AAT	503
Phe Glu Gly Ile Gly Tyr Ala Ser Gln Met Ile Val Ser Leu Leu Asn	
115 120 125	
GTC TAC TAC ATC GTT GTC CTG GCC TGG GCC CTC TTC TAC CTC TTC AGC	551
Val Tyr Tyr Ile Val Val Leu Ala Trp Ala Leu Phe Tyr Leu Phe Ser	
130 135 140	
AGC TTC ACC ACT GAC CTC CCC TGG GGT AGC TGC AGC CAC GAG TGG AAT	599
Ser Phe Thr Thr Asp Leu Pro Trp Gly Ser Cys Ser His Glu Trp Asn	
145 150 155	
ACA GAA AAC TGT GTG GAG TTC CAG AAA ACC AAC AAT TCC CTG AAT GTG	647
Thr Glu Asn Cys Val Glu Phe Gln Lys Thr Asn Asn Ser Leu Asn Val	
160 165 170	
ACT TCT GAG AAT GCC ACA TCC CCT GTC ATC GAG TTC TGG GAG AGG CGA	695
Thr Ser Glu Asn Ala Thr Ser Pro Val Ile Glu Phe Trp Glu Arg Arg	
175 180 185 190	

GTC CTG AAG ATC TCA GAT GGC ATC CAG CAC CTG GGG TCC CTG CGC TGG Val Leu Lys Ile Ser Asp Gly Ile Gln His Leu Gly Ser Leu Arg Trp 195 200 205	743
GAG CTG GTC CTG TGC CTC CTG CTT GCC TGG ATC ATC TGC TAT TTC TGC Glu Leu Val Leu Cys Leu Leu Leu Ala Trp Ile Ile Cys Tyr Phe Cys 210 215 220	791
ATC TGG AAA GGG GTC AAG TCC ACA GGC AAG GTG GTG TAC TTC ACA GCT Ile Trp Lys Gly Val Lys Ser Thr Gly Lys Val Val Tyr Phe Thr Ala 225 230 235	839
ACT TTC CCT TAC CTC ATG CTG GTG GTC CTG TTG ATC CGA GGA GTA ACA Thr Phe Pro Tyr Leu Met Leu Val Val Leu Leu Ile Arg Gly Val Thr 240 245 250	887
CTG CCT GGA GCA GCC CAG GGA ATT CAG TTT TAC CTG TAC CCC AAC ATC Leu Pro Gly Ala Ala Gln Gly Ile Gln Phe Tyr Leu Tyr Pro Asn Ile 255 260 265 270	935
ACA CGT CTG TGG GAT CCC CAG GTG TGG ATG GAT GCG GGC ACC CAG ATC Thr Arg Leu Trp Asp Pro Gln Val Trp Met Asp Ala Gly Thr Gln Ile 275 280 285	983
TTC TTC TCC TTT GCC ATC TGC CTG GGG TGC CTC ACG GCC CTG GGC AGC Phe Phe Ser Phe Ala Ile Cys Leu Gly Cys Leu Thr Ala Leu Gly Ser 290 295 300	1031
TAC AAC AAG TAC CAC AAC AAC TGC TAC AGG GAC TGC GTC GCC CTT TGC Tyr Asn Lys Tyr His Asn Asn Cys Tyr Arg Asp Cys Val Ala Leu Cys 305 310 315	1079
ATT CTC AAC AGC AGC ACC AGC TTC GTG GCC GGG TTT GCC ATC TTC TCC Ile Leu Asn Ser Ser Thr Ser Phe Val Ala Gly Phe Ala Ile Phe Ser 320 325 330	1127
ATC CTG GGC TTC ATG TCT CAG GAG CAG GGC GTA CCC ATA TCT GAG GTT Ile Leu Gly Phe Met Ser Gln Glu Gln Gly Val Pro Ile Ser Glu Val 335 340 345 350	1175
GCT GAA TCA GGC CCT GGC CTG GCA TTC ATC GCC TAC CCT CGA GCT GTG Ala Glu Ser Gly Pro Gly Leu Ala Phe Ile Ala Tyr Pro Arg Ala Val 355 360 365	1223
GTG ATG TTA CCT TTC TCG CCT TTG TGG GCC TGC TGT TTC TTC TTC ATG Val Met Leu Pro Phe Ser Pro Leu Trp Ala Cys Cys Phe Phe Phe Met 370 375 380	1271
GTG GTT CTC CTG GGA CTA GAC AGC CAG TTT GTG TGT GTA GAA AGC CTC Val Val Leu Leu Gly Leu Asp Ser Gln Phe Val Cys Val Glu Ser Leu 385 390 395	1319
GTG ACA GCG CTG GTG GAC ATG TAT CCC CGG GTG TTC CGT AAG AAG AAC Val Thr Ala Leu Val Asp Met Tyr Pro Arg Val Phe Arg Lys Lys Asn 400 405 410	1367
CGG AGG GAG ATT CTC ATC CTC ATC GTG TCT GTC GTC TCT TTC TTC ATC Arg Arg Glu Ile Leu Ile Leu Ile Val Ser Val Val Ser Phe Phe Ile 415 420 425 430	1415

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GGG CTC ATT ATG CTC ACA GAG GGC GGC ATG TAC GTG TTC CAG CTC TTC Gly Leu Ile Met Leu Thr Glu Gly Gly Met Tyr Val Phe Gln Leu Phe 435 440 445	1463
GAC TAC TAT GCG GCC AGT GGC ATG TGT CTT CTC TTT GTG GCC ATC TTT Asp Tyr Tyr Ala Ala Ser Gly Met Cys Leu Leu Phe Val Ala Ile Phe 450 455 460	1511
GAG TCC CTC TGT GTG GCT TGG GTT TAC GGA GCC AGC CGC TTC TAT GAC Glu Ser Leu Cys Val Ala Trp Val Tyr Gly Ala Ser Arg Phe Tyr Asp 465 470 475	1559
AAC ATT GAA GAT ATG ATT GGG TAC AAG CCG TGG CCT CTT ATC AAA TAC Asn Ile Glu Asp Met Ile Gly Tyr Lys Pro Trp Pro Leu Ile Lys Tyr 480 485 490	1607
TGT TGG CTC TTT TTC ACG CCA GCT GTG TGC CTG GCA ACC TTC CTG TTC Cys Trp Leu Phe Phe Thr Pro Ala Val Cys Leu Ala Thr Phe Leu Phe 495 500 505 510	1655
TCC CTG ATC AAA TAC ACG CCA CTG ACC TAC AAC AAG AAG TAC ACA TAT Ser Leu Ile Lys Tyr Thr Pro Leu Thr Tyr Asn Lys Lys Tyr Thr Tyr 515 520 525	1703
CCA TGG TGG GGG GAT GCC CTG GGG TGG CTC CTA GCT CTG TCC TCC ATG Pro Trp Trp Gly Asp Ala Leu Gly Trp Leu Leu Ala Leu Ser Ser Met 530 535 540	1751
GTC TGC ATT CCT GCC TGG AGC ATC TAC AAG CTC AGG ACT CTC AAG GGC Val Cys Ile Pro Ala Trp Ser Ile Tyr Lys Leu Arg Thr Leu Lys Gly 545 550 555	1799
CCA CTC AGA GAG AGA CTT CGC CAG CTC GTG TGC CCG GCT GAA GAC CTT Pro Leu Arg Glu Arg Leu Arg Gln Leu Val Cys Pro Ala Glu Asp Leu 560 565 570	1847
CCC CAG AAG AGC CAA CCA GAG CTG ACT TCT CCA GCG ACA CCG ATG ACG Pro Gln Lys Ser Gln Pro Glu Leu Thr Ser Pro Ala Thr Pro Met Thr 575 580 585 590	1895
TCC CTC CTC AGG CTC ACA GAA CTG GAG TCT AAC TGC T AGGGACGAGG Ser Leu Leu Arg Leu Thr Glu Leu Glu Ser Asn Cys 595 600	1942
CCTTTGACAC ACCTGCGAGT CTGTCTGTGG GGACAGCTAC AGACACAGAG GGCAGAACCA	2002
CCCCTCCGTG CTGGGGCAGA GAGACA	2028

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 602 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Asp Asn Arg Val Ser Gly Thr Thr Ser Asn Gly Glu Thr Lys Pro  
 1 5 10 15  
 Val Cys Pro Val Met Glu Lys Val Glu Glu Asp Gly Thr Leu Glu Arg  
 20 25 30  
 Glu Gln Trp Thr Asn Lys Met Glu Phe Val Leu Ser Val Ala Gly Glu  
 35 40 45  
 Ile Ile Gly Leu Gly Asn Val Trp Arg Phe Pro Tyr Leu Cys Tyr Lys  
 50 55 60  
 Asn Gly Gly Gly Ala Phe Phe Ile Pro Tyr Leu Ile Phe Leu Phe Thr  
 65 70 75 80  
 Cys Gly Ile Pro Val Phe Phe Leu Glu Thr Ala Leu Gly Gln Tyr Thr  
 85 90 95  
 Asn Gln Gly Gly Ile Thr Ala Trp Arg Lys Ile Cys Pro Ile Phe Glu  
 100 105 110  
 Gly Ile Gly Tyr Ala Ser Gln Met Ile Val Ser Leu Leu Asn Val Tyr  
 115 120 125  
 Tyr Ile Val Val Leu Ala Trp Ala Leu Phe Tyr Leu Phe Ser Ser Phe  
 130 135 140  
 Thr Thr Asp Leu Pro Trp Gly Ser Cys Ser His Glu Trp Asn Thr Glu  
 145 150 155 160  
 Asn Cys Val Glu Phe Gln Lys Thr Asn Asn Ser Leu Asn Val Thr Ser  
 165 170 175  
 Glu Asn Ala Thr Ser Pro Val Ile Glu Phe Trp Glu Arg Arg Val Leu  
 180 185 190  
 Lys Ile Ser Asp Gly Ile Gln His Leu Gly Ser Leu Arg Trp Glu Leu  
 195 200 205  
 Val Leu Cys Leu Leu Leu Ala Trp Ile Ile Cys Tyr Phe Cys Ile Trp  
 210 215 220  
 Lys Gly Val Lys Ser Thr Gly Lys Val Val Tyr Phe Thr Ala Thr Phe  
 225 230 235 240  
 Pro Tyr Leu Met Leu Val Val Leu Leu Ile Arg Gly Val Thr Leu Pro  
 245 250 255  
 Gly Ala Ala Gln Gly Ile Gln Phe Tyr Leu Tyr Pro Asn Ile Thr Arg  
 260 265 270  
 Leu Trp Asp Pro Gln Val Trp Met Asp Ala Gly Thr Gln Ile Phe Phe  
 275 280 285  
 Ser Phe Ala Ile Cys Leu Gly Cys Leu Thr Ala Leu Gly Ser Tyr Asn  
 290 295 300  
 Lys Tyr His Asn Asn Cys Tyr Arg Asp Cys Val Ala Leu Cys Ile Leu  
 305 310 315 320  
 Asn Ser Ser Thr Ser Phe Val Ala Gly Phe Ala Ile Phe Ser Ile Leu  
 325 330 335

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Gly Phe Met Ser Gln Glu Gln Gly Val Pro Ile Ser Glu Val Ala Glu  
 340 345 350  
 Ser Gly Pro Gly Leu Ala Phe Ile Ala Tyr Pro Arg Ala Val Val Met  
 355 360 365  
 Leu Pro Phe Ser Pro Leu Trp Ala Cys Cys Phe Phe Phe Met Val Val  
 370 375 380  
 Leu Leu Gly Leu Asp Ser Gln Phe Val Cys Val Glu Ser Leu Val Thr  
 385 390 395 400  
 Ala Leu Val Asp Met Tyr Pro Arg Val Phe Arg Lys Lys Asn Arg Arg  
 405 410 415  
 Glu Ile Leu Ile Leu Ile Val Ser Val Val Ser Phe Phe Ile Gly Leu  
 420 425 430  
 Ile Met Leu Thr Glu Gly Gly Met Tyr Val Phe Gln Leu Phe Asp Tyr  
 435 440 445  
 Tyr Ala Ala Ser Gly Met Cys Leu Leu Phe Val Ala Ile Phe Glu Ser  
 450 455 460  
 Leu Cys Val Ala Trp Val Tyr Gly Ala Ser Arg Phe Tyr Asp Asn Ile  
 465 470 475 480  
 Glu Asp Met Ile Gly Tyr Lys Pro Trp Pro Leu Ile Lys Tyr Cys Trp  
 485 490 495  
 Leu Phe Phe Thr Pro Ala Val Cys Leu Ala Thr Phe Leu Phe Ser Leu  
 500 505 510  
 Ile Lys Tyr Thr Pro Leu Thr Tyr Asn Lys Lys Tyr Thr Tyr Pro Trp  
 515 520 525  
 Trp Gly Asp Ala Leu Gly Trp Leu Leu Ala Leu Ser Ser Met Val Cys  
 530 535 540  
 Ile Pro Ala Trp Ser Ile Tyr Lys Leu Arg Thr Leu Lys Gly Pro Leu  
 545 550 555 560  
 Arg Glu Arg Leu Arg Gln Leu Val Cys Pro Ala Glu Asp Leu Pro Gln  
 565 570 575  
 Lys Ser Gln Pro Glu Leu Thr Ser Pro Ala Thr Pro Met Thr Ser Leu  
 580 585 590  
 Leu Arg Leu Thr Glu Leu Glu Ser Asn Cys  
 595 600

## (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1938 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: both
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: N

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(iv) ANTI-SENSE: N

(v) FRAGMENT TYPE: N-terminal

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: rat brain

(B) CLONE: rB8b

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 16..1897

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGCGGCAGGG CGGCC ATG ACT GCG GAG CAA GCG CTG CCC CTG GGC AAC GGG	51
Met Thr Ala Glu Gln Ala Leu Pro Leu Gly Asn Gly	
1 5 10	
AAG GCG GCC GAG GAG GCG CGA GGG TCC GAG GCG CTG GGC GGC GGC GGC	99
Lys Ala Ala Glu Glu Ala Arg Gly Ser Glu Ala Leu Gly Gly Gly Gly	
15 20 25	
GGG GGC GCG GCG GGG ACG CGC GAG GCG CGC GAC AAG GCG GTC CAC GAG	147
Gly Gly Ala Ala Gly Thr Arg Glu Ala Arg Asp Lys Ala Val His Glu	
30 35 40	
CGC GGT CAC TGG AAC AAC AAG GTG GAG TTC GTG TTG AGC GTA GCG GGA	195
Arg Gly His Trp Asn Asn Lys Val Glu Phe Val Leu Ser Val Ala Gly	
45 50 55 60	
GAG ATC ATC GGT CTG GGC AAC GTG TGG CGC TTC CCC TAC CTG TGC TAC	243
Glu Ile Ile Gly Leu Gly Asn Val Trp Arg Phe Pro Tyr Leu Cys Tyr	
65 70 75	
AAG AAC GGC GGA GGG GCA TTC CTG ATT CCT TAC GTG GTG TTT TTC ATC	291
Lys Asn Gly Gly Ala Phe Leu Ile Pro Tyr Val Val Phe Phe Ile	
80 85 90	
TGC TGT GGA ATC CCC GTC TTC TTC CTG GAA ACG GCT CTG GGG CAG TTC	339
Cys Cys Gly Ile Pro Val Phe Phe Leu Glu Thr Ala Leu Gly Gln Phe	
95 100 105	
ACG AGC GAG GGC GGC ATC ACG TGC TGG AGG AGA GTC TGT CCT TTA TTT	387
Thr Ser Glu Gly Gly Ile Thr Cys Trp Arg Arg Val Cys Pro Leu Phe	
110 115 120	
GAA GGC ATC GGC TAT GCA ACA CAG GTG ATC GAG GCG CAT CTC AAT GTC	435
Glu Gly Ile Gly Tyr Ala Thr Gln Val Ile Glu Ala His Leu Asn Val	
125 130 135 140	
TAC TAC ATC ATC ATC CTG GCG TGG GCC ATC TTC TAC TTA AGC AAC TGC	483
Tyr Tyr Ile Ile Ile Leu Ala Trp Ala Ile Phe Tyr Leu Ser Asn Cys	
145 150 155	
TTC ACC ACC GAG CTC CCC TGG GCC ACC TGT GGG CAT GAG TGG AAC ACA	531
Phe Thr Thr Glu Leu Pro Trp Ala Thr Cys Gly His Glu Trp Asn Thr	
160 165 170	
GAG AAA TGT GTG GAG TTC CAG AAG CTG AAC TTC AGC AAC TAC AGT CAT	579
Glu Lys Cys Val Glu Phe Gln Lys Leu Asn Phe Ser Asn Tyr Ser His	
175 180 185	

GTG TCC CTG CAG AAC GCA ACC TCC CCG GTC ATG GAG TTC TGG GAA CGC Val Ser Leu Gln Asn Ala Thr Ser Pro Val Met Glu Phe Trp Glu Arg 190 195 200	627
CGG GTC TTG GCT ATA TCT GAT GGC ATT GAA CAC ATC GGG AAC CTC CGA Arg Val Leu Ala Ile Ser Asp Gly Ile Glu His Ile Gly Asn Leu Arg 205 210 215 220	675
TGG GAG CTG GCA CTG TGT CTC CTG GCG GCT TGG ACC ATC TGC TAC TTC Trp Glu Leu Ala Leu Cys Leu Leu Ala Trp Thr Ile Cys Tyr Phe 225 230 235	723
TGC ATC TGG AAG GGT ACG AAG TCA ACT GGA AAG GTC GTG TAT GTC ACT Cys Ile Trp Lys Gly Thr Lys Ser Thr Gly Lys Val Val Tyr Val Thr 240 245 250	771
GCA ACC TTC CCC TAC ATC ATG CTG CTG ATC CTC CTG ATC CGA GGG GTC Ala Thr Phe Pro Tyr Ile Met Leu Leu Ile Leu Leu Ile Arg Gly Val 255 260 265	819
ACG TTG CCG GGT GCC TCG GAA GGC ATC AAG TTC TAC CTG TAC CCT GAC Thr Leu Pro Gly Ala Ser Glu Gly Ile Lys Phe Tyr Leu Tyr Pro Asp 270 275 280	867
CTC TCC CGG CTC TCT GAT CCA CAG GTG TGG GTG GAT GCT GGG ACG CAG Leu Ser Arg Leu Ser Asp Pro Gln Val Trp Val Asp Ala Gly Thr Gln 285 290 295 300	915
ATC TTT TTC TCC TAT GCC ATC TGC CTG GGC TGC CTG ACC GCT CTG GGG Ile Phe Phe Ser Tyr Ala Ile Cys Leu Gly Cys Leu Thr Ala Leu Gly 305 310 315	963
AGT TAC AAC AAC TAT AAC AAC AAC TGC TAC AGG GAC TGT ATT ATG CTC Ser Tyr Asn Asn Tyr Asn Asn Asn Cys Tyr Arg Asp Cys Ile Met Leu 320 325 330	1011
TGC TGT CTG AAC AGT GGC ACC AGC TTC GTG GCT GGG TTT GCT ATC TTC Cys Cys Leu Asn Ser Gly Thr Ser Phe Val Ala Gly Phe Ala Ile Phe 335 340 345	1059
TCA GTC CTG GGC TTC ATG GCG TAC GAG CAG GGC GTG CCT ATT GCT CAG Ser Val Leu Gly Phe Met Ala Tyr Glu Gln Gly Val Pro Ile Ala Glu 350 355 360	1107
GTG GCA GAA TCA GGT CCT GGA CTG GCT TTC ATC GCC TAC CCC AAG GCT Val Ala Glu Ser Gly Pro Gly Leu Ala Phe Ile Ala Tyr Pro Lys Ala 365 370 375 380	1155
GTC ACT ATG ATG CCC CTG TCC CCA TTG TGG GCC ACC CTG TTC TTC ATG Val Thr Met Met Pro Leu Ser Pro Leu Trp Ala Thr Leu Phe Phe Met 385 390 395	1203
ATG CTC ATC TTC CTG GGC CTG GAC AGT CAG TTT GTG TGT GTG GAG AGC Met Leu Ile Phe Leu Gly Leu Asp Ser Gln Phe Val Cys Val Glu Ser 400 405 410	1251
CTT GTG ACA GCC GTG GTT GAC ATG TAC CCC AAG GTC TTC CGG CGG GGC Leu Val Thr Ala Val Val Asp Met Tyr Pro Lys Val Phe Arg Arg Gly 415 420 425	1299



TAC CGG CGA GAA CTG CTC ATC CTG GCC CTG TCC ATT GTC TCT TAT TTC Tyr Arg Arg Glu Leu Leu Ile Leu Ala Leu Ser Ile Val Ser Tyr Phe 430 435 440	1347
CTA GGC CTG GTG ATG CTG ACA GAG GGA GGC ATG TAC ATT TTC CAG CTT Leu Gly Leu Val Met Leu Thr Glu Gly Gly Met Tyr Ile Phe Gln Leu 445 450 455 460	1395
TTT GAC TCA TAC GCC GCC AGT GGC ATG TGC TTG CTC TTC GTG GCC ATC Phe Asp Ser Tyr Ala Ala Ser Gly Met Cys Leu Leu Phe Val Ala Ile 465 470 475	1443
TTT GAG TGT GTC TGC ATC GGC TGG GTG TAT GGA AGT AAC AGG TTC TAT Phe Glu Cys Val Cys Ile Gly Trp Val Tyr Gly Ser Asn Arg Phe Tyr 480 485 490	1491
GAC AAT ATT GAG GAC ATG ATT GGA TAC CGG CCA CTG TCA CTC ATC AAG Asp Asn Ile Glu Asp Met Ile Gly Tyr Arg Pro Leu Ser Leu Ile Lys 495 500 505	1539
TGG TGC TGG AAA GTT GTG ACC CCT GGG ATC TGT GCG GGC ATC TTC ATC Trp Cys Trp Lys Val Val Thr Pro Gly Ile Cys Ala Gly Ile Phe Ile 510 515 520	1587
TTC TTT CTG GTC AAG TAC AAG CCG CTC AAG TAC AAC AAT GTG TAC ACA Phe Phe Leu Val Lys Tyr Lys Pro Leu Lys Tyr Asn Asn Val Tyr Thr 525 530 535 540	1635
TAT CCT GCT TGG GGC TAC GGC ATT GGC TGG CTC ATG GCT CTG TCC TCC Tyr Pro Ala Trp Gly Tyr Gly Ile Gly Trp Leu Met Ala Leu Ser Ser 545 550 555	1683
ATG CTG TGC ATC CCG CTC TGG ATC TTC ATC AAG CTG TGG AAG ACA GAG Met Leu Cys Ile Pro Leu Trp Ile Phe Ile Lys Leu Trp Lys Thr Glu 560 565 570	1731
GGC ACC CTG CCC GAG AAA TTA CAG AAG TTG ACA GTC CCC AGC GCT GAT Gly Thr Leu Pro Glu Lys Leu Gln Lys Leu Thr Val Pro Ser Ala Asp 575 580 585	1779
CTG AAA ATG AGG GGC AAG CTT GGG GCC AGC CCA CGG ATG GTG ACC GTT Leu Lys Met Arg Gly Lys Leu Gly Ala Ser Pro Arg Met Val Thr Val 590 595 600	1827
AAT GAC TGT GAG GCC AAG GTC AAA GGC GAC GGT ACC ATC TCT GCC ATC Asn Asp Cys Glu Ala Lys Val Lys Gly Asp Gly Thr Ile Ser Ala Ile 605 610 615 620	1875
ACA GAG AAG GAG ACG CAC TTC T GATCCCCGCC AGCCACTTGG ATGTGTCTCC Thr Glu Lys Glu Thr His Phe 625	1927
AGCCTTCCTT C	1938

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 627 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Met Thr Ala Glu Gln Ala Leu Pro Leu Gly Asn Gly Lys Ala Ala Glu
 1           5           10           15
Glu Ala Arg Gly Ser Glu Ala Leu Gly Gly Gly Gly Gly Ala Ala
      20           25           30
Gly Thr Arg Glu Ala Arg Asp Lys Ala Val His Glu Arg Gly His Trp
      35           40           45
Asn Asn Lys Val Glu Phe Val Leu Ser Val Ala Gly Glu Ile Ile Gly
      50           55           60
Leu Gly Asn Val Trp Arg Phe Pro Tyr Leu Cys Tyr Lys Asn Gly Gly
      65           70           75           80
Gly Ala Phe Leu Ile Pro Tyr Val Val Phe Phe Ile Cys Cys Gly Ile
      85           90           95
Pro Val Phe Phe Leu Glu Thr Ala Leu Gly Gln Phe Thr Ser Glu Gly
      100           105           110
Gly Ile Thr Cys Trp Arg Arg Val Cys Pro Leu Phe Glu Gly Ile Gly
      115           120           125
Tyr Ala Thr Gln Val Ile Glu Ala His Leu Asn Val Tyr Tyr Ile Ile
      130           135           140
Ile Leu Ala Trp Ala Ile Phe Tyr Leu Ser Asn Cys Phe Thr Thr Glu
      145           150           155           160
Leu Pro Trp Ala Thr Cys Gly His Glu Trp Asn Thr Glu Lys Cys Val
      165           170           175
Glu Phe Gln Lys Leu Asn Phe Ser Asn Tyr Ser His Val Ser Leu Gln
      180           185           190
Asn Ala Thr Ser Pro Val Met Glu Phe Trp Glu Arg Arg Val Leu Ala
      195           200           205
Ile Ser Asp Gly Ile Glu His Ile Gly Asn Leu Arg Trp Glu Leu Ala
      210           215           220
Leu Cys Leu Leu Ala Ala Trp Thr Ile Cys Tyr Phe Cys Ile Trp Lys
      225           230           235           240
Gly Thr Lys Ser Thr Gly Lys Val Val Tyr Val Thr Ala Thr Phe Pro
      245           250           255
Tyr Ile Met Leu Leu Ile Leu Leu Ile Arg Gly Val Thr Leu Pro Gly
      260           265           270
Ala Ser Glu Gly Ile Lys Phe Tyr Leu Tyr Pro Asp Leu Ser Arg Leu
      275           280           285
Ser Asp Pro Gln Val Trp Val Asp Ala Gly Thr Gln Ile Phe Phe Ser
      290           295           300

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Tyr Ala Ile Cys Leu Gly Cys Leu Thr Ala Leu Gly Ser Tyr Asn Asn  
 305 310 315 320  
 Tyr Asn Asn Asn Cys Tyr Arg Asp Cys Ile Met Leu Cys Cys Leu Asn  
 325 330 335  
 Ser Gly Thr Ser Phe Val Ala Gly Phe Ala Ile Phe Ser Val Leu Gly  
 340 345 350  
 Phe Met Ala Tyr Glu Gln Gly Val Pro Ile Ala Glu Val Ala Glu Ser  
 355 360 365  
 Gly Pro Gly Leu Ala Phe Ile Ala Tyr Pro Lys Ala Val Thr Met Met  
 370 375 380  
 Pro Leu Ser Pro Leu Trp Ala Thr Leu Phe Phe Met Met Leu Ile Phe  
 385 390 395 400  
 Leu Gly Leu Asp Ser Gln Phe Val Cys Val Glu Ser Leu Val Thr Ala  
 405 410 415  
 Val Val Asp Met Tyr Pro Lys Val Phe Arg Arg Gly Tyr Arg Arg Glu  
 420 425 430  
 Leu Leu Ile Leu Ala Leu Ser Ile Val Ser Tyr Phe Leu Gly Leu Val  
 435 440 445  
 Met Leu Thr Glu Gly Gly Met Tyr Ile Phe Gln Leu Phe Asp Ser Tyr  
 450 455 460  
 Ala Ala Ser Gly Met Cys Leu Leu Phe Val Ala Ile Phe Glu Cys Val  
 465 470 475 480  
 Cys Ile Gly Trp Val Tyr Gly Ser Asn Arg Phe Tyr Asp Asn Ile Glu  
 485 490 495  
 Asp Met Ile Gly Tyr Arg Pro Leu Ser Leu Ile Lys Trp Cys Trp Lys  
 500 505 510  
 Val Val Thr Pro Gly Ile Cys Ala Gly Ile Phe Ile Phe Phe Leu Val  
 515 520 525  
 Lys Tyr Lys Pro Leu Lys Tyr Asn Asn Val Tyr Thr Tyr Pro Ala Trp  
 530 535 540  
 Gly Tyr Gly Ile Gly Trp Leu Met Ala Leu Ser Ser Met Leu Cys Ile  
 545 550 555 560  
 Pro Leu Trp Ile Phe Ile Lys Leu Trp Lys Thr Glu Gly Thr Leu Pro  
 565 570 575  
 Glu Lys Leu Gln Lys Leu Thr Val Pro Ser Ala Asp Leu Lys Met Arg  
 580 585 590  
 Gly Lys Leu Gly Ala Ser Pro Arg Met Val Thr Val Asn Asp Cys Glu  
 595 600 605  
 Ala Lys Val Lys Gly Asp Gly Thr Ile Ser Ala Ile Thr Glu Lys Glu  
 610 615 620  
 Thr His Phe  
 625

## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 2093 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: both  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:  
 (A) ORGANISM: Taurine

(vii) IMMEDIATE SOURCE:  
 (A) LIBRARY: rat brain  
 (B) CLONE: rB16a

(ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 127..1989  
 (D) OTHER INFORMATION:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GCCACGCGC CGATCGCCGC CAATCCCGCC AGCCTCGGGC CGGGCCATCC GCTGTGGGCT	60
TAGCCACCCA GATGCAGAGC CAGTGCCACA GCCTCTTCAG AGGAGCCTCT CAAGCAAAAC	120
GAGGAG ATG GCC ACC AAG GAG AAG CTT CAA TGT CTG AAA GAC TTC CAC	168
Met Ala Thr Lys Glu Lys Leu Gln Cys Leu Lys Asp Phe His	
1 5 10	
AAA GAC ATC CTG AAG CCT TCT CCA GGG AAG AGC CCA GGC ACG CGG CCT	216
Lys Asp Ile Leu Lys Pro Ser Pro Gly Lys Ser Pro Gly Thr Arg Pro	
15 20 25 30	
GAG GAT GAG GCT GAT GGG AAG CCC CCT CAG AGG GAG AAG TGG TCC AGC	264
Glu Asp Glu Ala Asp Gly Lys Pro Pro Gln Arg Glu Lys Trp Ser Ser	
35 40 45	
AAG ATC GAC TTT GTG CTG TCT GTG GCC GGA GGC TTC GTG GGT TTG GGC	312
Lys Ile Asp Phe Val Leu Ser Val Ala Gly Gly Phe Val Gly Leu Gly	
50 55 60	
AAT GTC TGG CGT TTC CCG TAC CTC TGC TAC AAA AAT GGT GGA GGT GCA	360
Asn Val Trp Arg Phe Pro Tyr Leu Cys Tyr Lys Asn Gly Gly Gly Ala	
65 70 75	
TTC CTC ATA CCG TAT TTT ATT TTC CTG TTT GGG AGC GGC CTG CCT GTG	408
Phe Leu Ile Pro Tyr Phe Ile Phe Leu Phe Gly Ser Gly Leu Pro Val	
80 85 90	
TTT TTC CTG GAG GTC ATC ATA GGC CAG TAC ACC TCA GAA GGG GGC ATC	456
Phe Phe Leu Glu Val Ile Ile Gly Gln Tyr Thr Ser Glu Gly Gly Ile	
95 100 105 110	

ACC TGC TGG GAG AAG ATC TGC CCC TTG TTC TCT GGC ATT GGC TAC GCG Thr Cys Trp Glu Lys Ile Cys Pro Leu Phe Ser Gly Ile Gly Tyr Ala 115 120 125	504
TCC ATC GTC ATC GTG TCC CTC CTG AAT GTG TAC TAC ATC GTC ATC CTG Ser Ile Val Ile Val Ser Leu Leu Asn Val Tyr Tyr Ile Val Ile Leu 130 135 140	552
GCC TGG GCC ACA TAC TAC CTA TTC CAG TCT TTC CAG AAG GAT CTT CCC Ala Trp Ala Thr Tyr Tyr Leu Phe Gln Ser Phe Gln Lys Asp Leu Pro 145 150 155	600
TGG GCC CAC TGC AAC CAT AGC TGG AAC ACG CCA CAG TGC ATG GAG GAC Trp Ala His Cys Asn His Ser Trp Asn Thr Pro Gln Cys Met Glu Asp 160 165 170	648
ACC CTG CGT AGG AAC GAG AGT CAC TGG GTC TCC CTT AGC GCC GCC AAC Thr Leu Arg Arg Asn Glu Ser His Trp Val Ser Leu Ser Ala Ala Asn 175 180 185 190	696
TTC ACT TCG CCT GTG ATC GAG TTC TGG GAG CGC AAC GTG CTC AGC CTG Phe Thr Ser Pro Val Ile Glu Phe Trp Glu Arg Asn Val Leu Ser Leu 195 200 205	744
TCC TCC GGA ATC GAC CAC CCA GGC AGT CTG AAA TGG GAC CTC GCG CTC Ser Ser Gly Ile Asp His Pro Gly Ser Leu Lys Trp Asp Leu Ala Leu 210 215 220	792
TGC CTC CTC TTA GTC TGG CTC GTC TGT TTT TTC TGC ATC TGG AAG GGT Cys Leu Leu Leu Val Trp Leu Val Cys Phe Phe Cys Ile Trp Lys Gly 225 230 235	840
GTT CGG TCC ACA GGC AAG GTT GTC TAC TTC ACT GCT ACT TTC CCG TTT Val Arg Ser Thr Gly Lys Val Val Tyr Phe Thr Ala Thr Phe Pro Phe 240 245 250	888
GCC ATG CTT CTG GTG CTG CTG GTC CGT GGA CTG ACC CTG CCA GGT GCT Ala Met Leu Leu Val Leu Leu Val Arg Gly Leu Thr Leu Pro Gly Ala 255 260 265 270	936
GGT GAA GGC ATC AAA TTC TAC CTG TAC CCT AAC ATC AGC CGC CTT GAG Gly Glu Gly Ile Lys Phe Tyr Leu Tyr Pro Asn Ile Ser Arg Leu Glu 275 280 285	984
GAC CCA CAG GTG TGG ATC GAC GCT GGA ACT CAG ATA TTC TTT TCC TAC Asp Pro Gln Val Trp Ile Asp Ala Gly Thr Gln Ile Phe Phe Ser Tyr 290 295 300	1032
GCT ATC TGC CTG GGG GCC ATG ACC TCA CTG GGA AGC TAT AAC AAG TAC Ala Ile Cys Leu Gly Ala Met Thr Ser Leu Gly Ser Tyr Asn Lys Tyr 305 310 315	1080
AAG TAT AAC TCG TAC AGG GAC TGT ATG CTG CTG GGA TGC CTG AAC AGT Lys Tyr Asn Ser Tyr Arg Asp Cys Met Leu Leu Gly Cys Leu Asn Ser 320 325 330	1128
GGT ACC AGT TTT GTG TCT GGC TTC GCA ATT TTT TCC ATC CTG GGC TTC Gly Thr Ser Phe Val Ser Gly Phe Ala Ile Phe Ser Ile Leu Gly Phe 335 340 345 350	1176

ATG GCA CAA GAG CAA GGG GTG GAC ATT GCT GAT GTG GCT GAG TCA GGT Met Ala Gln Glu Gln Gly Val Asp Ile Ala Asp Val Ala Glu Ser Gly 355 360 365	1224
CCT GGC TTG GCC TTC ATT GCC TAC CCA AAA GCT GTG ACC ATG ATG CCG Pro Gly Leu Ala Phe Ile Ala Tyr Pro Lys Ala Val Thr Met Met Pro 370 375 380	1272
CTG CCC ACC TTT TGG TCC ATT CTG TTT TTT ATT ATG CTC CTC TTG CTT Leu Pro Thr Phe Trp Ser Ile Leu Phe Phe Ile Met Leu Leu Leu Leu 385 390 395	1320
GGA CTG GAC AGC CAG TTT GTT GAA GTC GAA GGA CAG ATC ACA TCC TTG Gly Leu Asp Ser Gln Phe Val Glu Val Glu Gly Gln Ile Thr Ser Leu 400 405 410	1368
GTT GAT CTT TAC CCG TCC TTC CTA AGG AAG GGT TAT CGT CGG GAA ATC Val Asp Leu Tyr Pro Ser Phe Leu Arg Lys Gly Tyr Arg Arg Glu Ile 415 420 425 430	1416
TTC ATT GCC ATC GTG TGC AGC ATC AGC TAC CTG CTG GGG CTG ACG ATG Phe Ile Ala Ile Val Cys Ser Ile Ser Tyr Leu Leu Gly Leu Thr Met 435 440 445	1464
GTG ACG GAG GGT GGC ATG TAT GTG TTT CAA CTC TTT GAC TAC TAT GCA Val Thr Glu Gly Gly Met Tyr Val Phe Gln Leu Phe Asp Tyr Tyr Ala 450 455 460	1512
GCT AGT GGT GTA TGC CTT TTG TGG GTC GCA TTC TTT GAA TGT TTT GTT Ala Ser Gly Val Cys Leu Leu Trp Val Ala Phe Phe Glu Cys Phe Val 465 470 475	1560
ATT GCC TGG ATA TAT GGC GGT GAT AAC TTA TAT GAC GGT ATT GAG GAC Ile Ala Trp Ile Tyr Gly Gly Asp Asn Leu Tyr Asp Gly Ile Glu Asp 480 485 490	1608
ATG ATC GGC TAT CGG CCT GGA CCC TGG ATG AAG TAC AGC TGG GCT GTC Met Ile Gly Tyr Arg Pro Gly Pro Trp Met Lys Tyr Ser Trp Ala Val 495 500 505 510	1656
ATC ACT CCA GCT CTC TGT GTT GGA TGT TTC ATC TTC TCT CTC GTC AAG Ile Thr Pro Ala Leu Cys Val Gly Cys Phe Ile Phe Ser Leu Val Lys 515 520 525	1704
TAT GTA CCC CTG ACC TAC AAC AAA GTC TAC CGG TAC CCT GAT TGG GCA Tyr Val Pro Leu Thr Tyr Asn Lys Val Tyr Arg Tyr Pro Asp Trp Ala 530 535 540	1752
ATC GGG CTG GGC TGG GGC CTG GCC CTT TCC TCC ATG GTG TGT ATC CCC Ile Gly Leu Gly Trp Gly Leu Ala Leu Ser Ser Met Val Cys Ile Pro 545 550 555	1800
TTG GTC ATT GTC ATC CTC CTC TGC CGG ACG GAG GGA CCG CTC CGC GTG Leu Val Ile Val Ile Leu Leu Cys Arg Thr Glu Gly Pro Leu Arg Val 560 565 570	1848
AGA ATC AAA TAC CTG ATA ACC CCC AGG GAG CCC AAC CGC TGG GCT GTG Arg Ile Lys Tyr Leu Ile Thr Pro Arg Glu Pro Asn Arg Trp Ala Val 575 580 585 590	1896

GAG CGT GAA GGG GCT ACG CCC TTT CAC TCC AGA GCA ACC CTC ATG AAC 1944  
 Glu Arg Glu Gly Ala Thr Pro Phe His Ser Arg Ala Thr Leu Met Asn  
                   595                                  600                                  605

GGT GCA CTC ATG AAA CCC AGT CAC GTC ATT GTG GAG ACC ATG ATG 1989  
 Gly Ala Leu Met Lys Pro Ser His Val Ile Val Glu Thr Met Met  
                   610                                  615                                  620

TGAGGTCCGG GCTGTGTGAC CGGCGCCGCT TTCCTGCCGT TTACTAACCT TAGATTCTCC 2049  
 TAGGACCAGG TTTACAGAGC TTTATATTTG TACTAGGATT TTTT 2093

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 621 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ala Thr Lys Glu Lys Leu Gln Cys Leu Lys Asp Phe His Lys Asp  
   1                  5                                  10                                  15

Ile Leu Lys Pro Ser Pro Gly Lys Ser Pro Gly Thr Arg Pro Glu Asp  
                   20                                  25                                  30

Glu Ala Asp Gly Lys Pro Pro Gln Arg Glu Lys Trp Ser Ser Lys Ile  
                   35                                  40                                  45

Asp Phe Val Leu Ser Val Ala Gly Gly Phe Val Gly Leu Gly Asn Val  
   50                                  55                                  60

Trp Arg Phe Pro Tyr Leu Cys Tyr Lys Asn Gly Gly Gly Ala Phe Leu  
   65                                  70                                  75                                  80

Ile Pro Tyr Phe Ile Phe Leu Phe Gly Ser Gly Leu Pro Val Phe Phe  
                   85                                  90                                  95

Leu Glu Val Ile Ile Gly Gln Tyr Thr Ser Glu Gly Gly Ile Thr Cys  
                   100                                  105                                  110

Trp Glu Lys Ile Cys Pro Leu Phe Ser Gly Ile Gly Tyr Ala Ser Ile  
   115                                  120                                  125

Val Ile Val Ser Leu Leu Asn Val Tyr Tyr Ile Val Ile Leu Ala Trp  
   130                                  135                                  140

Ala Thr Tyr Tyr Leu Phe Gln Ser Phe Gln Lys Asp Leu Pro Trp Ala  
   145                                  150                                  155                                  160

His Cys Asn His Ser Trp Asn Thr Pro Gln Cys Met Glu Asp Thr Leu  
                   165                                  170                                  175

Arg Arg Asn Glu Ser His Trp Val Ser Leu Ser Ala Ala Asn Phe Thr  
                   180                                  185                                  190

Ser Pro Val Ile Glu Phe Trp Glu Arg Asn Val Leu Ser Leu Ser Ser  
   195                                  200                                  205

Gly Ile Asp His Pro Gly Ser Leu Lys Trp Asp Leu Ala Leu Cys Leu  
 210 215 220  
 Leu Leu Val Trp Leu Val Cys Phe Phe Cys Ile Trp Lys Gly Val Arg  
 225 230 235 240  
 Ser Thr Gly Lys Val Val Tyr Phe Thr Ala Thr Phe Pro Phe Ala Met  
 245 250 255  
 Leu Leu Val Leu Leu Val Arg Gly Leu Thr Leu Pro Gly Ala Gly Glu  
 260 265 270  
 Gly Ile Lys Phe Tyr Leu Tyr Pro Asn Ile Ser Arg Leu Glu Asp Pro  
 275 280 285  
 Gln Val Trp Ile Asp Ala Gly Thr Gln Ile Phe Phe Ser Tyr Ala Ile  
 290 295 300  
 Cys Leu Gly Ala Met Thr Ser Leu Gly Ser Tyr Asn Lys Tyr Lys Tyr  
 305 310 315 320  
 Asn Ser Tyr Arg Asp Cys Met Leu Leu Gly Cys Leu Asn Ser Gly Thr  
 325 330 335  
 Ser Phe Val Ser Gly Phe Ala Ile Phe Ser Ile Leu Gly Phe Met Ala  
 340 345 350  
 Gln Glu Gln Gly Val Asp Ile Ala Asp Val Ala Glu Ser Gly Pro Gly  
 355 360 365  
 Leu Ala Phe Ile Ala Tyr Pro Lys Ala Val Thr Met Met Pro Leu Pro  
 370 375 380  
 Thr Phe Trp Ser Ile Leu Phe Phe Ile Met Leu Leu Leu Leu Gly Leu  
 385 390 395 400  
 Asp Ser Gln Phe Val Glu Val Glu Gly Gln Ile Thr Ser Leu Val Asp  
 405 410 415  
 Leu Tyr Pro Ser Phe Leu Arg Lys Gly Tyr Arg Arg Glu Ile Phe Ile  
 420 425 430  
 Ala Ile Val Cys Ser Ile Ser Tyr Leu Leu Gly Leu Thr Met Val Thr  
 435 440 445  
 Glu Gly Gly Met Tyr Val Phe Gln Leu Phe Asp Tyr Tyr Ala Ala Ser  
 450 455 460  
 Gly Val Cys Leu Leu Trp Val Ala Phe Phe Glu Cys Phe Val Ile Ala  
 465 470 475 480  
 Trp Ile Tyr Gly Gly Asp Asn Leu Tyr Asp Gly Ile Glu Asp Met Ile  
 485 490 495  
 Gly Tyr Arg Pro Gly Pro Trp Met Lys Tyr Ser Trp Ala Val Ile Thr  
 500 505 510  
 Pro Ala Leu Cys Val Gly Cys Phe Ile Phe Ser Leu Val Lys Tyr Val  
 515 520 525  
 Pro Leu Thr Tyr Asn Lys Val Tyr Arg Tyr Pro Asp Trp Ala Ile Gly  
 530 535 540



Leu Gly Trp Gly Leu Ala Leu Ser Ser Met Val Cys Ile Pro Leu Val  
 545 550 555 560  
 Ile Val Ile Leu Leu Cys Arg Thr Glu Gly Pro Leu Arg Val Arg Ile  
 565 570 575  
 Lys Tyr Leu Ile Thr Pro Arg Glu Pro Asn Arg Trp Ala Val Glu Arg  
 580 585 590  
 Glu Gly Ala Thr Pro Phe His Ser Arg Ala Thr Leu Met Asn Gly Ala  
 595 600 605  
 Leu Met Lys Pro Ser His Val Ile Val Glu Thr Met Met  
 610 615 620

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1051 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: both  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: N

## (iv) ANTI-SENSE: N

## (vii) IMMEDIATE SOURCE:

- (A) LIBRARY: human heart, human brain  
 (B) CLONE: hHE7a,hS3a

## (ix) FEATURE:

- (A) NAME/KEY: CDS  
 (B) LOCATION: 1..739  
 (D) OTHER INFORMATION:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CTG GCT TTC ATC GCT TAC CCG CGG GCT GTG GTG ATG CTG CCC TTC TCT	48
Leu Ala Phe Ile Ala Tyr Pro Arg Ala Val Val Met Leu Pro Phe Ser	
1 5 10 15	
CCT CTC TGG GCC TGC TGT TTC TTC TTC ATG GTC GTT CTC CTG GGA CTG	96
Pro Leu Trp Ala Cys Cys Phe Phe Phe Met Val Val Leu Leu Gly Leu	
20 25 30	
GAT AGC CAG TTT GTG TGT GTA GAA AGC CTG GTG ACA GCG CTG GTG GAC	144
Asp Ser Gln Phe Val Cys Val Glu Ser Leu Val Thr Ala Leu Val Asp	
35 40 45	
ATG TAC CCT CAC GTG TTC CGC AAG AAG AAC CGG AGG GAA GTC CTC ATC	192
Met Tyr Pro His Val Phe Arg Lys Lys Asn Arg Arg Glu Val Leu Ile	
50 55 60	
CTT GGA GTA TCT GTC GTC TCC TTC CTT GTG GGG CTG ATC ATG CTC ACA	240
Leu Gly Val Ser Val Val Ser Phe Leu Val Gly Leu Ile Met Leu Thr	
65 70 75 80	

GAG GGC GGA ATG TAC GTG TTC CAG CTC TTT GAC TAC TAT GCG GCC AGT Glu Gly Gly Met Tyr Val Phe Gln Leu Phe Asp Tyr Tyr Ala Ala Ser 85 90 95	288
GGC ATG TGC CTC CTG TTC GTG GCC ATC TTC GAG TCC CTC TGT GTG GCT Gly Met Cys Leu Leu Phe Val Ala Ile Phe Glu Ser Leu Cys Val Ala 100 105 110	336
TGG GTT TAC GGA GCC AAG CGC TTC TAC GAC AAC ATC GAA GAC ATG ATT Trp Val Tyr Gly Ala Lys Arg Phe Tyr Asp Asn Ile Glu Asp Met Ile 115 120 125	384
GGG TAC AGG CCA TGG CCT CTT ATC AAA TAC TGT TGG CTC TTC CTC ACA Gly Tyr Arg Pro Trp Pro Leu Ile Lys Tyr Cys Trp Leu Phe Leu Thr 130 135 140	432
CCA GCT GTG TGC ACA GCC ACC TTT CTC TTC TCC CTG ATA AAG TAC ACT Pro Ala Val Cys Thr Ala Thr Phe Leu Phe Ser Leu Ile Lys Tyr Thr 145 150 155 160	480
CCG CTG ACC TAC AAC AAG AAG TAC ACG TAC CCG TGG TGG GGC GAT GCC Pro Leu Thr Tyr Asn Lys Lys Tyr Thr Tyr Pro Trp Trp Gly Asp Ala 165 170 175	528
CTG GGC TGG CTC CTG GCT CTG TCC TCC ATG GTC TGC ATT CCT GCC TGG Leu Gly Trp Leu Leu Ala Leu Ser Ser Met Val Cys Ile Pro Ala Trp 180 185 190	576
AGC CTC TAC AGA CTC GGA ACC CTC AAG GGC CCC TTC AGA GAG AGA ATC Ser Leu Tyr Arg Leu Gly Thr Leu Lys Gly Pro Phe Arg Glu Arg Ile 195 200 205	624
CGT CAG CTC ATG TGC CCA GCC GAG GAC CTG CCC CAG CGG AAC CCA GCA Arg Gln Leu Met Cys Pro Ala Glu Asp Leu Pro Gln Arg Asn Pro Ala 210 215 220	672
GGA CCC TCG GCT CCC GCC ACC CCC AGG ACC TCA CTG CTC AGA CTC ACA Gly Pro Ser Ala Pro Ala Thr Pro Arg Thr Ser Leu Leu Arg Leu Thr 225 230 235 240	720
GAG CTA GAG TCT CAC TGC T AGGGGGCAGG CCCTTGGATG GTGCCTGTGT Glu Leu Glu Ser His Cys 245	769
GCCTGGCCTT GGGGATGGCT GTGGAGGGAA CGTGGCAGAA GCAGCCCCAT GTGCTTCCCT	829
GCCCCCGACC TGGAGTGGAT AAGACAAGAG GGGTATTTTG GAGTCCACCT GCTGAGCTGG	889
AGGCCTCCCA CTGCAACTTT TCAGCTCAGG GGTGTGTGAA CAGATGTGAA AGGCCAGTGC	949
CAAGAGTGTC CCTCTGAGAC CCTTGGGAAG CTGGGTGGGG GCTGGTAGGT GGGGCGAGAC	1009
TTGCTGGCTT CGGGCCCTCT CATCCTTCAT TCCATTAAAT CC	1051

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 246 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```

Leu Ala Phe Ile Ala Tyr Pro Arg Ala Val Val Met Leu Pro Phe Ser
 1           5           10           15
Pro Leu Trp Ala Cys Cys Phe Phe Phe Met Val Val Leu Leu Gly Leu
          20           25           30
Asp Ser Gln Phe Val Cys Val Glu Ser Leu Val Thr Ala Leu Val Asp
          35           40           45
Met Tyr Pro His Val Phe Arg Lys Lys Asn Arg Arg Glu Val Leu Ile
          50           55           60
Leu Gly Val Ser Val Val Ser Phe Leu Val Gly Leu Ile Met Leu Thr
          65           70           75           80
Glu Gly Gly Met Tyr Val Phe Gln Leu Phe Asp Tyr Tyr Ala Ala Ser
          85           90           95
Gly Met Cys Leu Leu Phe Val Ala Ile Phe Glu Ser Leu Cys Val Ala
          100          105          110
Trp Val Tyr Gly Ala Lys Arg Phe Tyr Asp Asn Ile Glu Asp Met Ile
          115          120          125
Gly Tyr Arg Pro Trp Pro Leu Ile Lys Tyr Cys Trp Leu Phe Leu Thr
          130          135          140
Pro Ala Val Cys Thr Ala Thr Phe Leu Phe Ser Leu Ile Lys Tyr Thr
          145          150          155          160
Pro Leu Thr Tyr Asn Lys Lys Tyr Thr Tyr Pro Trp Trp Gly Asp Ala
          165          170          175
Leu Gly Trp Leu Leu Ala Leu Ser Ser Met Val Cys Ile Pro Ala Trp
          180          185          190
Ser Leu Tyr Arg Leu Gly Thr Leu Lys Gly Pro Phe Arg Glu Arg Ile
          195          200          205
Arg Gln Leu Met Cys Pro Ala Glu Asp Leu Pro Gln Arg Asn Pro Ala
          210          215          220
Gly Pro Ser Ala Pro Ala Thr Pro Arg Thr Ser Leu Leu Arg Leu Thr
          225          230          235          240
Glu Leu Glu Ser His Cys
          245

```

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1991 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: both
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: human brain

(B) CLONE: hGAT-3

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 35..1930

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGCCGGGCCG GCGCAGGAGG CAGCCAGCGC GGCC ATG ACG GCG GAG AAG GCG	52
Met Thr Ala Glu Lys Ala	
1 5	
CTG CCC CTG GGC AAT GGG AAG GCT GCT GAG GAG GCG CGG GAG TCC GAG	100
Leu Pro Leu Gly Asn Gly Lys Ala Ala Glu Glu Ala Arg Glu Ser Glu	
10 15 20	
GCG CCG GGT GGC GGC TGC AGC AGC GGG GGC GCG GCG CCC GCG CGC CAC	148
Ala Pro Gly Gly Gly Cys Ser Ser Gly Gly Ala Ala Pro Ala Arg His	
25 30 35	
CCG CGC GTC AAG CGC GAC AAG GCG GTC CAC GAG CGC GGC CAC TGG AAC	196
Pro Arg Val Lys Arg Asp Lys Ala Val His Glu Arg Gly His Trp Asn	
40 45 50	
AAC AAG GTG GAG TTC GTG CTG AGC GTG GCC GGG GAG ATC ATT GGG CTG	244
Asn Lys Val Glu Phe Val Leu Ser Val Ala Gly Glu Ile Ile Gly Leu	
55 60 65 70	
GGC AAC GTG TGG CGC TTC CCC TAC CTG TGC TAC AAG AAC GGA GGA GGG	292
Gly Asn Val Trp Arg Phe Pro Tyr Leu Cys Tyr Lys Asn Gly Gly Gly	
75 80 85	
GCA TTC CTG ATT CCC TAC GTG GTG TTT TTT ATT TGC TGT GGA ATT CCT	340
Ala Phe Leu Ile Pro Tyr Val Val Phe Phe Ile Cys Cys Gly Ile Pro	
90 95 100	
GTT TTT TTC CTG GAG ACA GCT CTG GGG CAG TTC ACA AGT GAA GGT GGC	388
Val Phe Phe Leu Glu Thr Ala Leu Gly Gln Phe Thr Ser Glu Gly Gly	
105 110 115	
ATT ACG TGT TGG AGG AAA GTT TGC CCT TTA TTT GAA GGC ATT GGC TAT	436
Ile Thr Cys Trp Arg Lys Val Cys Pro Leu Phe Glu Gly Ile Gly Tyr	
120 125 130	
GCA ACA CAG GTG ATT GAG GCC CAT CTG AAT GTG TAC TAC ATC ATC ATC	484
Ala Thr Gln Val Ile Glu Ala His Leu Asn Val Tyr Tyr Ile Ile Ile	
135 140 145 150	
CTG GCA TGG GCC ATT TTT TAC CTG AGC AAC TGC TTC ACT ACT GAG CTA	532
Leu Ala Trp Ala Ile Phe Tyr Leu Ser Asn Cys Phe Thr Thr Glu Leu	
155 160 165	

CCC TGG GCT ACC TGT GGG CAT GAG TGG AAC ACA GAG AAT TGT GTG GAG Pro Trp Ala Thr Cys Gly His Glu Trp Asn Thr Glu Asn Cys Val Glu 170 175 180	580
TTC CAG AAA CTG AAT GTG AGC AAC TAC AGC CAT GTG TCT CTG CAG AAT Phe Gln Lys Leu Asn Val Ser Asn Tyr Ser His Val Ser Leu Gln Asn 185 190 195	628
GCC ACC TCC CCT GTC ATG GAG TTT TGG GAG CAC CGG GTC CTG GCC ATC Ala Thr Ser Pro Val Met Glu Phe Trp Glu His Arg Val Leu Ala Ile 200 205 210	676
TCT GAC GGG ATC GAG CAC ATC GGG AAC CTT CGC TGG GAG CTG GCC TTG Ser Asp Gly Ile Glu His Ile Gly Asn Leu Arg Trp Glu Leu Ala Leu 215 220 225 230	724
TGT CTC TTG GCA GCC TGG ACC ATC TGT TAC TTC TGT ATC TGG AAG GGG Cys Leu Leu Ala Ala Trp Thr Ile Cys Tyr Phe Cys Ile Trp Lys Gly 235 240 245	772
ACC AAG TCT ACA GGA AAG GTT GTA TAC GTG ACT GCG ACA TTC CCC TAC Thr Lys Ser Thr Gly Lys Val Val Tyr Val Thr Ala Thr Phe Pro Tyr 250 255 260	820
ATC ATG CTG CTG ATC CTC CTG ATA CGA GGG GTC ACG TTG CCC GGG GCC Ile Met Leu Leu Ile Leu Leu Ile Arg Gly Val Thr Leu Pro Gly Ala 265 270 275	868
TCA GAG GGC ATC AAG TTC TAC TTG TAC CCT GAC CTC TCC CGG CTC TCC Ser Glu Gly Ile Lys Phe Tyr Leu Tyr Pro Asp Leu Ser Arg Leu Ser 280 285 290	916
GAC CCC CAG GTC TGG GTA GAT GCT GGA ACG CAG ATC TTT TTC TCC TAT Asp Pro Gln Val Trp Val Asp Ala Gly Thr Gln Ile Phe Phe Ser Tyr 295 300 305 310	964
GCC ATT TGC CTG GGC TGT CTG ACC GCT CTG GGA AGT TAT AAC AAT TAT Ala Ile Cys Leu Leu Cys Leu Thr Ala Leu Gly Ser Tyr Asn Asn Tyr 315 320 325	1012
AAC AAC AAC TGC TAC AGG GAC TGC ATC ATG CTC TGT TGC CTG AAC AGC Asn Asn Asn Cys Tyr Arg Asp Cys Ile Met Leu Cys Cys Leu Asn Ser 330 335 340	1060
GGC ACC AGC TTC GTG GCT GGG TTT GCC ATC TTC TCA GTC CTG GGT TTT Gly Thr Ser Phe Val Ala Gly Phe Ala Ile Phe Ser Val Leu Gly Phe 345 350 355	1108
ATG GCG TAC GAG CAG GGG GTA CCC ATT GCT GAG GTG GCA GAG TCA GGC Met Ala Tyr Glu Gln Gly Val Pro Ile Ala Glu Val Ala Glu Ser Gly 360 365 370	1156
CCC GGC CTG GCC TTT ATT GCG TAC CCC AAG GCG GTC ACC ATG ATG CCT Pro Gly Leu Ala Phe Ile Ala Tyr Pro Lys Ala Val Thr Met Met Pro 375 380 385 390	1204
CTC TCC CCG CTG TGG GCC ACC TTG TTC TTC ATG ATG CTC ATC TTC CTG Leu Ser Pro Leu Trp Ala Thr Leu Phe Phe Met Met Leu Ile Phe Leu 395 400 405	1252

GGC CTG GAC AGC CAG TTT GTG TGT GTG GAA AGC CTG GTG ACC GCC GTG Gly Leu Asp Ser Gln Phe Val Cys Val Glu Ser Leu Val Thr Ala Val 410 415 420	1300
GTG GAC ATG TAC CCC AAG GTT TTC CGG AGG GGT TAC CGG CGG GAG CTG Val Asp Met Tyr Pro Lys Val Phe Arg Arg Gly Tyr Arg Glu Leu 425 430 435	1348
CTC ATC CTA GCC TTG TCT GTT ATC TCC TAT TTT CTG GGC CTC GTG ATG Leu Ile Leu Ala Leu Ser Val Ile Ser Tyr Phe Leu Gly Leu Val Met 440 445 450	1396
TTA ACA GAG GGT GGC ATG TAC ATC TTC CAG CTC TTT GAC TCC TAT GCC Leu Thr Glu Gly Gly Met Tyr Ile Phe Gln Leu Phe Asp Ser Tyr Ala 455 460 465 470	1444
GCC AGT GGG ATG TGC CTT CTC TTC GTG GCC ATC TTT GAG TGC ATC TGC Ala Ser Gly Met Cys Leu Leu Phe Val Ala Ile Phe Glu Cys Ile Cys 475 480 485	1492
ATC GGC TGG GTG TAT GGA AGC AAC CGG TTC TAT GAT AAC ATT GAA GAC Ile Gly Trp Val Tyr Gly Ser Asn Arg Phe Tyr Asp Asn Ile Glu Asp 490 495 500	1540
ATG ATT GGC TAC CGG CCA CCG TCG CTC ATT AAG TGG TGC TGG ATG ATC Met Ile Gly Tyr Arg Pro Pro Ser Leu Ile Lys Trp Cys Trp Met Ile 505 510 515	1588
ATG ACC CCT GGG ATC TGC GCG GGG ATC TTC ATC TTC TTC TTG ATC AAG Met Thr Pro Gly Ile Cys Gly Ile Phe Ile Phe Leu Ile Lys 520 525 530	1636
TAC AAG CCA CTC AAG TAC AAC AAC ATC TAC ACC TAC CCA GCC TGG GGC Tyr Lys Pro Leu Lys Tyr Asn Asn Ile Tyr Thr Tyr Pro Ala Trp Gly 535 540 545 550	1684
TAT GGC ATT GGC TGG CTC ATG GCC CTG TCC TCC ATG CTC TGC ATC CCG Tyr Gly Ile Gly Trp Leu Met Ala Leu Ser Ser Met Leu Cys Ile Pro 555 560 565	1732
CTC TGG ATC TGC ATC ACA GTG TGG AAG ACG GAG GGG ACA CTG CCC GAG Leu Trp Ile Cys Ile Thr Val Trp Lys Thr Glu Gly Thr Leu Pro Glu 570 575 580	1780
AAA CTC CAG AAG TTG ACG ACC CCC AGC ACA GAT CTG AAA ATG CGG GGC Lys Leu Gln Lys Leu Thr Thr Pro Ser Thr Asp Leu Lys Met Arg Gly 585 590 595	1828
AAG CTT GGG GTG AGC CCA CGG ATG GTG ACA GTT AAT GAC TGT GAT GCC Lys Leu Gly Val Ser Pro Arg Met Val Thr Val Asn Asp Cys Asp Ala 600 605 610	1876
AAA CTC AAG AGT GAC GGG ACC ATC GCA GCC ATC ACA GAG AAG GAG ACG Lys Leu Lys Ser Asp Gly Thr Ile Ala Ala Ile Thr Glu Lys Glu Thr 615 620 625 630	1924
CAC TTC TGAGCGGCCA CCAGCCATCT GGGGCTCTTC TTCCTTTCTT CCCCCGTGT His Phe	1980
ATGTAAATGA A	1991

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 632 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

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Met Thr Ala Glu Lys Ala Leu Pro Leu Gly Asn Gly Lys Ala Ala Glu
 1           5           10           15
Glu Ala Arg Glu Ser Glu Ala Pro Gly Gly Gly Cys Ser Ser Gly Gly
 20           25           30
Ala Ala Pro Ala Arg His Pro Arg Val Lys Arg Asp Lys Ala Val His
 35           40           45
Glu Arg Gly His Trp Asn Asn Lys Val Glu Phe Val Leu Ser Val Ala
 50           55           60
Gly Glu Ile Ile Gly Leu Gly Asn Val Trp Arg Phe Pro Tyr Leu Cys
 65           70           75           80
Tyr Lys Asn Gly Gly Gly Ala Phe Leu Ile Pro Tyr Val Val Phe Phe
 85           90           95
Ile Cys Cys Gly Ile Pro Val Phe Phe Leu Glu Thr Ala Leu Gly Gln
100          105          110
Phe Thr Ser Glu Gly Gly Ile Thr Cys Trp Arg Lys Val Cys Pro Leu
115          120          125
Phe Glu Gly Ile Gly Tyr Ala Thr Gln Val Ile Glu Ala His Leu Asn
130          135          140
Val Tyr Tyr Ile Ile Ile Leu Ala Trp Ala Ile Phe Tyr Leu Ser Asn
145          150          155          160
Cys Phe Thr Thr Glu Leu Pro Trp Ala Thr Cys Gly His Glu Trp Asn
165          170          175
Thr Glu Asn Cys Val Glu Phe Gln Lys Leu Asn Val Ser Asn Tyr Ser
180          185          190
His Val Ser Leu Gln Asn Ala Thr Ser Pro Val Met Glu Phe Trp Glu
195          200          205
His Arg Val Leu Ala Ile Ser Asp Gly Ile Glu His Ile Gly Asn Leu
210          215          220
Arg Trp Glu Leu Ala Leu Cys Leu Leu Ala Ala Trp Thr Ile Cys Tyr
225          230          235          240
Phe Cys Ile Trp Lys Gly Thr Lys Ser Thr Gly Lys Val Val Tyr Val
245          250          255
Thr Ala Thr Phe Pro Tyr Ile Met Leu Leu Ile Leu Leu Ile Arg Gly
260          265          270

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Val Thr Leu Pro Gly Ala Ser Glu Gly Ile Lys Phe Tyr Leu Tyr Pro  
 275 280 285  
 Asp Leu Ser Arg Leu Ser Asp Pro Gln Val Trp Val Asp Ala Gly Thr  
 290 295 300  
 Gln Ile Phe Phe Ser Tyr Ala Ile Cys Leu Gly Cys Leu Thr Ala Leu  
 305 310 315 320  
 Gly Ser Tyr Asn Asn Tyr Asn Asn Asn Cys Tyr Arg Asp Cys Ile Met  
 325 330 335  
 Leu Cys Cys Leu Asn Ser Gly Thr Ser Phe Val Ala Gly Phe Ala Ile  
 340 345 350  
 Phe Ser Val Leu Gly Phe Met Ala Tyr Glu Gln Gly Val Pro Ile Ala  
 355 360 365  
 Glu Val Ala Glu Ser Gly Pro Gly Leu Ala Phe Ile Ala Tyr Pro Lys  
 370 375 380  
 Ala Val Thr Met Met Pro Leu Ser Pro Leu Trp Ala Thr Leu Phe Phe  
 385 390 395 400  
 Met Met Leu Ile Phe Leu Gly Leu Asp Ser Gln Phe Val Cys Val Glu  
 405 410 415  
 Ser Leu Val Thr Ala Val Val Asp Met Tyr Pro Lys Val Phe Arg Arg  
 420 425 430  
 Gly Tyr Arg Arg Glu Leu Leu Ile Leu Ala Leu Ser Val Ile Ser Tyr  
 435 440 445  
 Phe Leu Gly Leu Val Met Leu Thr Glu Gly Gly Met Tyr Ile Phe Gln  
 450 455 460  
 Leu Phe Asp Ser Tyr Ala Ala Ser Gly Met Cys Leu Leu Phe Val Ala  
 465 470 475 480  
 Ile Phe Glu Cys Ile Cys Ile Gly Trp Val Tyr Gly Ser Asn Arg Phe  
 485 490 495  
 Tyr Asp Asn Ile Glu Asp Met Ile Gly Tyr Arg Pro Pro Ser Leu Ile  
 500 505 510  
 Lys Trp Cys Trp Met Ile Met Thr Pro Gly Ile Cys Ala Gly Ile Phe  
 515 520 525  
 Ile Phe Phe Leu Ile Lys Tyr Lys Pro Leu Lys Tyr Asn Asn Ile Tyr  
 530 535 540  
 Thr Tyr Pro Ala Trp Gly Tyr Gly Ile Gly Trp Leu Met Ala Leu Ser  
 545 550 555 560  
 Ser Met Leu Cys Ile Pro Leu Trp Ile Cys Ile Thr Val Trp Lys Thr  
 565 570 575  
 Glu Gly Thr Leu Pro Glu Lys Leu Gln Lys Leu Thr Thr Pro Ser Thr  
 580 585 590  
 Asp Leu Lys Met Arg Gly Lys Leu Gly Val Ser Pro Arg Met Val Thr  
 595 600 605



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Val Asn Asp Cys Asp Ala Lys Leu Lys Ser Asp Gly Thr Ile Ala Ala  
610 615 620

Ile Thr Glu Lys Glu Thr His Phe  
625 630

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What is claimed is:

1. An isolated nucleic acid molecule encoding a mammalian GABA transporter.
- 5 2. A nucleic acid molecule of claim 1, wherein the nucleic acid molecule encodes a rat GABA transporter.
- 10 3. A nucleic acid molecule of claim 1, wherein the nucleic acid encodes a human GABA transporter.
4. An isolated DNA molecule of claim 1, wherein the nucleic acid encodes a murine transporter.
- 15 5. An isolated nucleic acid molecule encoding a mammalian taurine transporter.
6. A nucleic acid molecule of claim 5, wherein the nucleic acid encodes a rat taurine transporter.
- 20 7. A nucleic acid molecule of claim 5, wherein the nucleic acid molecule encodes a human taurine transporter.
- 25 8. A nucleic acid molecule of claim 5, wherein the nucleic acid molecule encodes a murine taurine transporter.
- 30 9. An isolated nucleic acid molecule of claim 1, wherein the nucleic acid molecule is a DNA molecule.
- 35 10. An DNA molecule of claim 9, wherein the DNA molecule is a cDNA molecule.

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11. An isolated nucleic acid molecule of claim 5,  
wherein the nucleic acid molecule is a DNA molecule.
12. A DNA molecule of claim 11, wherein the DNA molecule  
is a cDNA molecule.
13. An isolated nucleic acid molecule of claim 1,  
wherein the nucleic acid molecule has been so  
mutated as to be incapable of normal transporter  
activity, and not expressing native GABA  
transporter.
14. An isolated nucleic acid molecule of claim 5,  
wherein the nucleic acid molecule has been so  
mutated as to be incapable of normal transporter  
activity, and not expressing native taurine  
transporter.
15. A method for isolating a nucleic acid molecule  
encoding a human taurine transporter by nucleic acid  
sequence homology using natural sequences or  
artificial sequences, the sequences of which are  
derived from sequences in Figures 1A, 1B, 1C 10A or  
10B.
16. An isolated mammalian GABA transporter protein.
17. The mammalian GABA transporter protein of claim 16,  
wherein the protein is a human GABA transporter.
18. An isolated mammalian taurine transporter protein.
19. The mammalian transporter protein of claim 18,  
wherein the protein is a rat taurine transporter.

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20. The mammalian transporter protein of claim 18,  
wherein the transporter human taurine transporter.
21. A vector comprising the DNA molecule of claim 9.
22. A plasmid comprising the vector of claim 21.
23. A vector comprising the DNA molecule of claim 11.
24. A plasmid comprising the vector of claim 23.
25. A vector of claim 21 adapted for expression in a  
bacterial cell which comprises the regulatory  
elements necessary for expression of the DNA in the  
bacterial cell so located relative to the DNA  
encoding the transporter as to permit expression  
thereof.
26. A vector of claim 21 adapted for expression in a  
yeast cell which comprises the regulatory elements  
necessary for expression of the DNA in the yeast  
cell so located relative to the DNA encoding the  
transporter as to permit expression thereof.
27. A vector of claim 21 adapted for expression in a  
mammalian cell which comprises the regulatory  
elements necessary for expression of the DNA in the  
mammalian cell so located relative to the DNA  
encoding the transporter as to permit expression  
thereof.
28. A vector of claim 23 adapted for expression in a  
bacteria cell which comprises the regulatory  
elements necessary for expression of the DNA in the

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bacterial cell so located relative to the DNA encoding the transporter as to permit expression thereof.

- 5        29. A vector of claim 23 adapted for expression in a yeast cell which comprises the regulatory elements necessary for expression of the DNA in the yeast cell so located relative to the DNA encoding the transporter as to permit expression thereof.
- 10       30. A vector of claim 23 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the DNA encoding the transporter as to permit expression thereof.
- 15       32. A plasmid of claim 22 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the DNA encoding the GABA transporter as to permit expression thereof.
- 20       33. A plasmid of claim 24 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the DNA encoding the taurine transporter as to permit expression thereof.
- 25       34. A plasmid designated pEVJB-rB14b (ATCC Accession No.        ).
- 30       35. A plasmid designated pEVJB-rB8b (ATCC Accession No.        ).
- 35

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36. A plasmid designated pEVJB-rB16a (ATCC Accession No. ).
37. A plasmid designated pcEXV-hGAT-3.
- 5 38. A plasmid designated pBluescript-hHe7a.
39. A plasmid designated pBluescript-hS3a.
- 10 40. A mammalian cell comprising the plasmid of claim 22.
41. A mammalian cell comprising the plasmid of claim 24.
- 15 42. The mammalian cell of claim 40, wherein the mammalian cell is a Cos7 cell.
43. The mammalian cell of claim 41, wherein the mammalian cell is a Cos7 cell.
- 20 44. A Cos7 cell comprising the plasmid of claim 32.
45. A Cos7 cell comprising the plasmid of claim 33.
- 25 46. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a mammalian GABA transporter.
- 30 47. A nucleic acid probe of claim 46 wherein the nucleic acid probe is capable of specifically hybridizing with a human GABA transporter.
- 35 48. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of

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specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a mammalian taurine transporter.

- 5      49. A nucleic acid probe of claim 48, wherein the nucleic acid probe is capable of specifically hybridizing with a human taurine transporter.
- 10      50. The nucleic acid probe of claims 46, wherein the nucleic acid is DNA.
51. The nucleic acid probe of claims 48, wherein the nucleic acid is DNA.
- 15      52. An antisense oligonucleotide having a sequence capable of binding specifically to an mRNA molecule encoding a mammalian GABA transporter so as to prevent translation of the mRNA molecule.
- 20      53. The antisense oligonucleotide of claim 52, wherein the antisense oligonucleotide is capable of binding specifically to an mRNA molecule encoding a human GABA transporter so as to prevent translation of the mRNA encoding a human GABA transporter.
- 25      54. An antisense oligonucleotide having a sequence capable of binding specifically to an mRNA molecule encoding a mammalian taurine transporter so as to prevent translation of the mRNA molecule.
- 30      55. The antisense oligonucleotide of claim 54 having a sequence capable of binding specifically to an mRNA molecule encoding a rat taurine transporter so as to prevent translation of the mRNA molecule.
- 35

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56. The antisense oligonucleotide of claim 54, wherein the antisense oligonucleotide is capable of binding specifically to an mRNA molecule encoding a human taurine transporter so as to prevent translation of the mRNA.
57. An antisense oligonucleotide having a sequence capable of binding specifically to the cDNA molecule of claim 10.
58. An antisense oligonucleotide capable of specifically hybridizing to the cDNA molecule of claim 12.
59. An antisense oligonucleotide of claim 52, comprising chemical analogues of nucleotides.
60. An antisense oligonucleotide of claim 54, comprising chemical analogues of nucleotides.
61. A monoclonal antibody directed to a mammalian GABA transporter.
62. A monoclonal antibody of claim 61, wherein the monoclonal antibody is directed to a human GABA transporter.
63. A monoclonal antibody directed to a mammalian taurine transporter.
64. A monoclonal antibody of claim 63, wherein the monoclonal antibody is directed to a human taurine transporter.
65. A monoclonal antibody of claim 63, wherein the monoclonal antibody is directed to a rat taurine



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receptor.

- 5 66. A monoclonal antibody of claim 61, directed to an epitope of a mammalian cell-surface GABA transporter and having an amino acid sequence substantially the same as an amino acid sequence for a cell-surface epitope of the mammalian GABA transporter.
- 10 67. A monoclonal antibody of claim 63, directed to an epitope of a mammalian cell-surface taurine transporter and having an amino acid sequence substantially the same as an amino acid sequence for a cell-surface epitope of the mammalian taurine transporter.
- 15 68. A pharmaceutical composition comprising an effective amount of the oligonucleotide of claim 52 effective to reduce expression of a mammalian GABA transporter by passing through a cell membrane and binding specifically with mRNA encoding a mammalian GABA transporter in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane.
- 20 69. A pharmaceutical composition comprising an effective amount of the oligonucleotide of claim 54 effective to reduce expression of a mammalian taurine transporter by passing through a cell membrane and binding specifically with mRNA encoding a mammalian taurine transporter in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane.
- 25 30 35

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70. A pharmaceutical composition of claim 68, wherein the oligonucleotide is coupled to a substance which inactivates mRNA.
- 5 71. A pharmaceutical composition of claim 69, wherein the oligonucleotide is coupled to a substance which inactivates mRNA.
- 10 72. A pharmaceutical composition of claim 70, wherein the substance which inactivates mRNA is a ribozyme.
73. A pharmaceutical composition of claim 71, wherein the substance which inactivates mRNA is a ribozyme.
- 15 74. A pharmaceutical composition of claim 70, wherein the pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane comprises a structure which binds to a transporter specific for a selected cell type and is thereby taken up by
- 20 cells of the selected cell type.
75. A pharmaceutical composition of claim 71, wherein the pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane comprises
- 25 a structure which binds to a transporter specific for a selected cell type and is thereby taken up by cells of the selected cell type.
- 30 76. A pharmaceutical composition comprising an amount of a substance effective to alleviate the abnormalities resulting from overexpression of a mammalian GABA transporter and a pharmaceutically acceptable carrier.
- 35 77. A pharmaceutical composition comprising an amount of

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a substance effective to alleviate the abnormalities resulting from overexpression of a mammalian taurine transporter and a pharmaceutically acceptable carrier.

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78. A pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of GABA transporter and a pharmaceutically acceptable carrier.

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79. A pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of taurine transporter and a pharmaceutically acceptable carrier.

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80. A pharmaceutical composition which comprises an amount of the antibody of claim 61 effective to block binding of naturally occurring substrates to the GABA transporter and a pharmaceutically acceptable carrier.

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81. A pharmaceutical composition which comprises an amount of the antibody of claim 63 effective to block binding of naturally occurring substrates to the taurine transporter and a pharmaceutically acceptable carrier.

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83. A transgenic nonhuman mammal which comprises the isolated nucleic acid molecule of claim 1.

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84. A transgenic nonhuman mammal which comprises the isolated nucleic acid molecule of claim 5.

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85. A transgenic nonhuman mammal which comprises the

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isolated nucleic acid molecule of claim 13.

86. A transgenic nonhuman mammal which comprises the isolated nucleic acid molecule of claim 14.

5

87. A transgenic nonhuman mammal whose genome comprises DNA encoding a mammalian GABA transporter so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a GABA transporter and which hybridizes to mRNA encoding a GABA transporter thereby reducing its translation.

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88. A transgenic nonhuman mammal whose genome comprises DNA encoding a mammalian taurine transporter so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a taurine transporter and which hybridizes to mRNA encoding a taurine transporter thereby reducing its translation.

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89. The transgenic nonhuman mammal of claim 83 wherein the DNA encoding a mammalian GABA transporter further comprises an inducible promoter.

25

90. The transgenic nonhuman mammal of claim 84, wherein the DNA encoding a mammalian taurine transporter further comprises an inducible promoter.

30

91. The transgenic nonhuman mammal of claim 83, wherein the DNA encoding a mammalian GABA transporter additionally comprises tissue specific regulatory elements.

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92. The transgenic nonhuman mammal of claim 84, wherein the DNA encoding a mammalian taurine transporter

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additionally comprises tissue specific regulatory elements.

- 5 95. A transgenic animal of claim 83, wherein the transgenic animal is a mouse.
96. A transgenic animal of claim 84, wherein the transgenic animal is a mouse.
- 10 97. A transgenic nonhuman mammal whose genome comprises DNA complementary to DNA encoding a mammalian GABA transporter so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding the transporter and which hybridizes to
- 15 mRNA encoding the transporter thereby preventing its translation.
- 20 98. A transgenic nonhuman mammal whose genome comprises DNA complementary to DNA encoding a mammalian taurine transporter so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding the transporter and which hybridizes to mRNA encoding the transporter thereby preventing its translation.
- 25 99. A method for determining whether a substrate is capable of binding to a mammalian GABA transporter which comprises contacting the mammalian cell of claim 40 with the substrate under suitable
- 30 conditions to permit binding of the substrate to the transporter, detecting the presence of any substrate bound to the mammalian transporter, and the presence of bound substrate indicating that the substrate is capable of binding to the mammalian transporter.
- 35

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100. The method of claim 99, wherein the transporter is a human GABA transporter.
- 5 101. A method for determining whether a substrate is capable of binding to a mammalian taurine transporter which comprises contacting the mammalian cell of claim 41 with the substrate under suitable conditions to permit binding of the substrate to the transporter, detecting the presence of any substrate bound to the mammalian transporter, and the presence of bound substrate indicating that the substrate is capable of binding to the mammalian transporter.
- 10 102. The method of claim 101, wherein the mammalian transporter is a human taurine transporter.
- 15 103. The method of claim 99, wherein the mammalian cell is nonneuronal in origin.
- 20 104. The method of claim 101, wherein the mammalian cell is a non-neuronal in origin.
- 25 105. The non-neuronal cell of claim 103, wherein the cell is a Cos7 cell.
106. The non-neuronal cell of claim 104, wherein the cell is a Cos7 cell.
- 30 106. A substrate detected by the method of claim 99.
107. A substrate detected by the method of claim 101.
- 35 108. A method of screening drugs to identify drugs which specifically interact with, and bind to, a mammalian

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GABA transporter expressed on the surface of the cell, which comprises contacting a mammalian cell of claim 40 with a plurality of drugs under conditions that permit binding of drugs to the transporter, determining those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, a mammalian GABA transporter.

- 10 109. A method of claim of screening drugs to identify drugs which specifically interact with, and bind to, a mammalian taurine transporter expressed on the surface of the cell, which comprises contacting a mammalian cell of claim 41 with a plurality of drugs under conditions that permit binding of drugs to the transporter, determining those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, a mammalian GABA transporter.
- 15 110. The method of claim 108, wherein the mammalian cell is nonneuronal on origin.
- 20 111. The method of claim 109, wherein the mammalian cell is nonneuronal in origin.
- 25 112. The mammalian cell of claim 110, wherein the cell is a COS7 cell.
- 30 113. The mammalian cell of claim 111, wherein the cell is a COS7 cell.
- 35 114. A pharmaceutical composition of a drug identified by the method of claims 108 or 109.

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115. A method of detecting expression of a cell-surface transporter which comprises obtaining total mRNA from the cell, contacting the mRNA so obtained with the nucleic acid probe of claim 46, under hybridizing conditions, detecting the presence of any mRNA hybridized to the probe, the presence of mRNA hybridized to the probe indicating expression of the cell-surface transporter and thereby detecting the expression of the transporter by the cell.
116. A method of detecting expression of a cell-surface transporter which comprises obtaining total mRNA from the cell, contacting the mRNA so obtained with the nucleic acid probe of claim 48, under hybridizing conditions, detecting the presence of any mRNA hybridized to the probe, the presence of mRNA hybridized to the probe indicating expression of the cell-surface transporter and thereby detecting the expression of the transporter by the cell.
117. A method of treating abnormalities in a subject, wherein the abnormality is alleviated by the reduced expression of a GABA transporter which comprises administering to a subject an effective amount of the pharmaceutical composition of claim 68, effective to reduce expression of the GABA transporter in the subject.
118. A method of treating abnormalities in a subject, wherein the abnormality is alleviated by the reduced expression of a taurine transporter which comprises administering to a subject an effective amount of the pharmaceutical composition of claim 69,



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effective to reduce expression of the taurine transporter in the subject.

- 5           119. A method of treating an abnormal condition related to an excess of GABA transporter activity which comprises administering to a subject an effective amount of the pharmaceutical composition of claim 68, effective to reduce expression of the GABA transporter in the subject.
- 10           120. A method of treating an abnormal condition related to an excess of taurine transporter activity which comprises administering to a subject an effective amount of the pharmaceutical composition of claim
- 15           69, effective to reduce expression of the taurine transporter in the subject.
- 20           121. The method of claims 119 or 120 wherein the abnormal condition is epilepsy.
122. The method of claim 119, wherein the abnormal condition is generalized anxiety.
- 25           123. The method of claim 120, wherein the abnormal condition is migraine.
124. The method of claim 120, wherein the abnormal condition is ischemia.
- 30           125. A method of treating abnormalities which are alleviated by reduction of expression of a mammalian GABA transporter which comprises administering to a subject an amount of the pharmaceutical composition of claim 80 effective to block binding of naturally
- 35           occurring substrates to the GABA transporter and

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thereby alleviate abnormalities resulting from overexpression of a mammalian GABA transporter.

5 126. A method of treating abnormalities which are alleviated by reduction of expression of a mammalian taurine transporter which comprises administering to a subject an amount of the pharmaceutical composition of claim 81 effective to block binding of naturally occurring substrates to the taurine transporter and thereby alleviate abnormalities resulting from overexpression of a mammalian taurine transporter.

15 127. A method of treating an abnormal condition related to an excess of GABA transporter activity which comprises administering to a subject an amount of the pharmaceutical composition of claim 80 effective to block binding of naturally occurring substrates to the GABA transporter and thereby alleviate the abnormal condition.

25 128. A method of treating an abnormal condition related to an excess of taurine transporter activity which comprises administering to a subject an amount of the pharmaceutical composition of claim 81 effective to block binding of naturally occurring substrates to the taurine transporter and thereby alleviate the abnormal condition.

30 129. The method of claims 127 or 128, wherein the abnormal condition is epilepsy.

35 130. The method of claim 127, wherein the abnormal condition is generalized anxiety.

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131. The method of claim 128, wherein the abnormal condition is migraine.
132. The method of claim 128, wherein the abnormal condition is ischemia.
133. A method of detecting the presence of a mammalian GABA transporter on the surface of a cell which comprises contacting the cell with the antibody of claim 61 under conditions permitting binding of the antibody to the transporter, detecting the presence of any antibody bound to the cell, and thereby detecting the presence of a mammalian GABA transporter on the surface of the cell.
134. A method of detecting the presence of a mammalian taurine transporter on the surface of a cell which comprises contacting the cell with the antibody of claim 63 under conditions permitting binding of the antibody to the transporter, detecting the presence of any antibody bound to the cell, and thereby detecting the presence of a mammalian taurine transporter on the surface of the cell.
135. A method of determining the physiological effects of expressing varying levels of mammalian GABA transporters which comprises producing a transgenic nonhuman animal whose levels of mammalian GABA transporter expression are varied by use of an inducible promoter which regulates mammalian GABA transporter expression.
136. A method of determining the physiological effects of expressing varying levels of mammalian taurine transporters which comprises producing a transgenic

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nonhuman animal whose levels of mammalian taurine transporter expression are varied by use of an inducible promoter which regulates mammalian taurine transporter expression.

- 5
137. A method of determining the physiological effects of expressing varying levels of mammalian GABA transporters which comprises producing a panel of transgenic nonhuman animals each expressing a  
10 different amount of mammalian GABA transporter.
138. A method of determining the physiological effects of expressing varying levels of mammalian taurine transporters which comprises producing a panel of  
15 transgenic nonhuman animals each expressing a different amount of mammalian taurine transporter.
139. A method for identifying a substance capable of alleviating the abnormalities resulting from overexpression of a mammalian GABA transporter comprising administering a substance to the  
20 transgenic nonhuman mammal of claim 47 and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of  
25 overexpression of a mammalian GABA transporter.
140. A method for identifying a substance capable of alleviating the abnormalities resulting from overexpression of a mammalian taurine transporter comprising administering a substance to the  
30 transgenic nonhuman mammal of claim 48 and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of  
35

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overexpression of a mammalian taurine transporter.

- 5 141. A method for treating the abnormalities resulting from overexpression of a mammalian GABA transporter which comprises administering to a subject an amount of the pharmaceutical composition of claim 83 effective to alleviate the abnormalities resulting from overexpression of a mammalian GABA transporter.
- 10 142. A method for treating the abnormalities resulting from overexpression of a mammalian taurine transporter which comprises administering to a subject an amount of the pharmaceutical composition of claim 84 effective to alleviate the abnormalities resulting from overexpression of a mammalian taurine transporter.
- 15 143. A method for identifying a substance capable of alleviating the abnormalities resulting from underexpression of a mammalian GABA transporter comprising administering the substance to the transgenic nonhuman mammal of either of claims 83, 85, or 87 and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of underexpression of a mammalian transporter.
- 20 144. A method for identifying a substance capable of alleviating the abnormalities resulting from underexpression of a mammalian taurine transporter comprising administering the substance to the transgenic nonhuman mammal of either of claims 84, 86, or 88 and determining whether the substance alleviates the physical and behavioral abnormalities
- 25 30 35

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displayed by the transgenic nonhuman mammal as a result of underexpression of a mammalian transporter.

- 5      145. A method for treating the abnormalities resulting from underexpression of a mammalian transporter which comprises administering to a subject an amount of the pharmaceutical composition of claims 78 or 79 effective to alleviate the abnormalities resulting from underexpression of a mammalian transporter.
- 10
146. A method for diagnosing a predisposition to a disorder associated with the expression of a specific mammalian transporter allele which comprises:
- 15
- a. obtaining DNA of subjects suffering from the disorder;
- 20
- b. performing a restriction digest of the DNA with a panel of restriction enzymes;
- c. electrophoretically separating the resulting DNA fragments on a sizing gel;
- 25
- d. contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a mammalian transporter and labelled with a detectable marker;
- 30
- e. detecting labelled bands which have hybridized to the DNA encoding a mammalian transporter labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder;
- 35

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f. preparing DNA obtained for diagnosis by steps a-e; and

g. comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

147. The method of claim 96 wherein a disorder associated with the expression of a specific mammalian transporter allele is diagnosed.

148. A method of preparing the isolated transporter of claims 16 or 18 which comprises:

a. inducing cells to express transporter;

b. recovering the transporter from the resulting cells; and

c. purifying the transporter so recovered.

149. A method of preparing the isolated transporter of claims 16 or 18 which comprises:

a. inserting nucleic acid encoding transporter in a suitable vector;

b. inserting the resulting vector in a suitable host cell;

c. recovering the transporter produced by the

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resulting cell; and

d. purifying the transporter so recovered.

5 150. A method for preparing membranes comprising a GABA transporter which comprises:

a. inserting nucleic acid encoding the GABA transporter in a suitable vector;

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b. inserting the resulting vector in a suitable host cell;

c. preparing a cell lysate; and

15

d. isolating membranes from the resulting cell lysate.

20 151. A method for preparing membranes comprising a taurine transporter which comprises:

a. inserting nucleic acid encoding the taurine transporter in a suitable vector;

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b. inserting the resulting vector in a suitable host cell;

c. preparing a cell lysate; and

30

d. isolating membranes from the resulting cell lysate.

152. A method for isolating vesicles comprising the GABA transporter which comprises:

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- a. inserting nucleic acid encoding the GABA transporter in a suitable vector;
- b. inserting the resulting vector in a suitable host cell;
- c. preparing a cell lysate; and
- d. isolating vesicles from the resulting cell lysate.

153. A method for isolating vesicles comprising a taurine transporter which comprises:

- a. inserting nucleic acid encoding the taurine transporter in a suitable vector;
- b. inserting the resulting vector in a suitable host cell; and
- c. preparing a cell lysate; and
- d. isolating vesicles from the resulting cell lysate.

154. A method for determining whether a compound is capable of binding to a mammalian GABA transporter which comprises contacting a preparation of the isolated membranes of claim 150 with the compound under suitable conditions to permit binding of the compound to the transporter, detecting the presence of any compound bound to the transporter, and the presence of bound compound indicating that the compound is capable of binding to the mammalian GABA transporter.

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155. A method for determining whether a compound is capable of binding to a mammalian taurine transporter which comprises contacting a preparation of isolated membranes of claim 151 with the compound under suitable conditions to permit binding of the compound to the taurine transporter, detecting the presence of any compound bound to the taurine transporter, and the presence of bound compound indicating that the compound is capable of binding to the mammalian taurine transporter.
156. A method for determining whether a compound is capable of binding to a mammalian GABA transporter which comprises contacting a preparation of isolated vesicles of claim 152 with the compound under suitable conditions to permit binding of the compound to the transporter, detecting the presence of any compound bound to the transporter, and the presence of bound compound indicating that the compound is capable of binding to the mammalian GABA transporter.
157. A method for determining whether a compound is capable of binding to a mammalian taurine transporter which comprises contacting the preparation of isolated vesicles of claim 153 with the compound under suitable conditions to permit binding of the compound to the taurine transporter, detecting the presence of any compound bound to the taurine transporter, and the presence of bound compound indicating that the compound is capable of binding to the mammalian taurine transporter.
158. A method for identifying a compound which enhances

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or decreases GABA transporter activity which comprises contacting a preparation of membrane vesicles of claim 152 with the compound under suitable conditions to permit binding of the compound, and detecting an increase or decrease in GABA transporter activity.

5

159. A method for identifying a compound which enhances or decreases taurine transporter activity which comprises contacting a preparation of membrane vesicles of claim 153 with the compound under suitable conditions to permit binding of the compound, and detecting an increase or decrease in taurine transporter activity.

10

15

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FIGURE 1A

-120 -100 -80  
GGCAGCGAACACAAGCGCATCCGGTAGAACGGAAAGAACAGGAATTGCAGAGTGACTTCA  
-60 -40 -20  
AGTCTCCATACGATTCTACTACCCGGGTGACGGCAGTGACTCGACAGAGTAGCGGCTGCAG  
0 20 40  
GTGGGATGGATAACAGGGTCTCGGGAACGACCAGTAATGGAGAGACAAAGCCAGTGTGTC  
M D N R V S G T T S N G E T K P V C P  
60 80 100  
CAGTCATGGAGAAGGTGGAGGAAGACGGTACCTTGAACGGGAGCAATGGACCAACAAGA  
V M E K V E E D G T L E R E Q W T N K M  
120 140 160  
TGGAGTTCGTACTGTCTCAGTGGCGGGAGAGATCATTGGCTTAGGCAACGTCTGGAGGTTTC  
E F V L S V A G E I I G L G N V W R F P  
180 200 220  
CCTATCTCTGCTACAAGAACGGGGGAGGTGCCTTCTTTATTCCTACCTCATCTTCCTAT  
Y L C Y K N G G G A F F I P Y L I F L F  
240 260 280  
TTACCTGTGGCATTCTCTGTCTTCTTCTGAGACAGCGCTTGGCCAGTACACCAACCAGG  
T C G I P V F F L E T A L G Q Y T N Q G  
300 320 340  
GAGGCATCACAGCCTGGAGGAAATCTGTCCCATCTTCGAGGGCATCGGCTATGCCTCAC  
G I T A W R K I C P I F E G I G Y A S Q  
360 380 400  
AGATGATCGTCAGCCTTCTCAATGTCTACTACATCGTTGTCCTGGCCTGGGCCCTCTTCT  
M I V S L L N V Y Y I V V L A W A L F Y  
420 440 460  
ACCTCTTCAGCAGCTTCACCACTGACCTCCCCTGGGGTAGCTGCAGCCACGAGTGGAATA  
L F S S F T T D L P W G S C S H E W N T

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480 500 520  
CAGAAACTGTGTGGAGTTCCAGAAAACCAACAATTCCTGAATGTGACTTCTGAGAATG  
E N C V E F Q K T N N S L N V T S E N A

540 560 580  
CCACATCCCCTGTCTCGAGTTCTGGGAGAGGCGAGTCCTGAAGATCTCAGATGGCATCC  
T S P V I E F W E R R V L K I S D G I Q

600 620 640  
AGCACCTGGGGTCCCTGCGCTGGGAGCTGGTCCTGTGCCTCCTGCTTGCCTGGATCATCT  
H L G S L R W E L V L C L L L A W I I C

660 680 700  
GCTATTTCTGCATCTGGAAAGGGGTCAAGTCCACAGGCAAGGTGGTGTACTTCACAGCTA  
Y F C I W K G V K S T G K V V Y F T A T

720 740 760  
CTTTCCTTACCTCATGCTGGTGGTCCTGTTGATCCGAGGAGTAACACTGCCTGGAGCAG  
F P Y L M L V V L L I R G V T L P G A A

780 800 820  
CCCAGGGAATTCAGTTTACCTGTACCCCAACATCACACGTCTGTGGGATCCCCAGGTGT  
Q G I Q F Y L Y P N I T R L W D P Q V W

840 860 880  
GGATGGATGCGGGCACCAGATCTTCTTCTCCTTTGCCATCTGCCTGGGGTGCCTCACGG  
M D A G T Q I F F S F A I C L G C L T A

900 920 940  
CCCTGGGCAGCTACAACAAGTACCACAACAAGTCTACAGGGACTGCGTCGCCCCTTGCA  
L G S Y N K Y H N N C Y R D C V A L C I

960 980 1000  
TTCTCAACAGCAGCACCAGCTTCGTGGCCGGGTTTGCCATCTTCTCCATCCTGGGCTTCA  
L N S S T S F V A G F A I F S I L G F M

1020 1040 1060  
TGTCTCAGGAGCAGGGCGTACCCATATCTGAGGTGCTGAATCAGGCCCTGGCCTGGCAT  
S Q E Q G V P I S E V A E S G P G L A F

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1080 1100 1120  
TCATCGCCTACCCTCGAGCTGTGGTGTATGTTACCTTTCTCGCCTTTGTGGGCCTGCTGTT  
I A Y P R A V V M L P F S P L W A C C F

1140 1160 1180  
TCTTCTTCATGGTGGTTCTCCTGGGACTAGACAGCCAGTTTGTGTGTGTAGAAAGCCTCG  
F F M V V L L G L D S Q F V C V E S L V

1200 1220 1240  
TGACAGCGCTGGTGGACATGTATCCCCGGGTGTTCCGTAAGAAGAACCGGAGGGAGATTC  
T A L V D M Y P R V F R K K N R R E I L

1260 1280 1300  
TCATCCTCATCGTGTCTGTCTCTCTTTCTTCATCGGGCTCATTATGCTCACAGAGGGCG  
I L I V S V V S F F I G L I M L T E G G

1320 1340 1360  
GCATGTACGTGTTCCAGCTCTTCGACTACTATGCGGCCAGTGGCATGTGTCTTCTCTTTG  
M Y V F Q L F D Y Y A A S G M C L L F V

1380 1400 1420  
TGGCCATCTTTGAGTCCCTCTGTGTGGCTTGGGTTTACGGAGCCAGCCGCTTCTATGACA  
A I F E S L C V A W V Y G A S R F Y D N

1440 1460 1480  
ACATTGAAGATATGATTGGGTACAAGCCGTGGCCTCTTATCAAATACTGTTGGCTCTTTT  
I E D M I G Y K P W P L I K Y C W L F F

1500 1520 1540  
TCACGCCAGCTGTGTGCCTGGCAACCTTCTGTTCTCCCTGATCAAATACAGCCCACTGA  
T P A V C L A T F L F S L I K Y T P L T

1560 1580 1600  
CCTACAACAAGAAGTACACATATCCATGGTGGGGGGATGCCCTGGGGTGGCTCCTAGCTC  
Y N K K Y T Y P W W G D A L G W L L A L

1620 1640 1660  
TGTCTCCATGGTCTGCATTCTGCTGGAGCATCTACAAGCTCAGGACTCTCAAGGGCC  
S S M V C I P A W S I Y K L R T L K G P

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1680 1700 1720  
CACTCAGAGAGAGACTTCGCCAGCTCGTGTGCCCCGGCTGAAGACCTTCCCCAGAAGAGCC  
L R E R L R Q L V C P A E D L P Q K S Q

1740 1760 1780  
AACCAGAGCTGACTTCTCCAGCGACACCGATGACGTCCCTCCTCAGGCTCACAGAACTGG  
P E L T S P A T P M T S L L R L T E L E

1800 1820 1840  
AGTCTAACTGCTAGGGACGAGGCCTTTGACACACCTGCGAGTCTGTCTGTGGGGACAGCT  
S N C

1860 1880 1900  
ACAGACACAGAGGGCAGAACCACCCCTCCGTGCTGGGGCAGAGAGACA

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FIGURE 1B

-10 10 30  
GGCGGCAGGGCGGCCATGACTGCGGAGCAAGCGCTGCCCCTGGGCAACGGGAAGGCGGCC  
M T A E Q A L P L G N G K A A

50 70 90  
GAGGAGGCGCGAGGGTCCGAGGCGCTGGGCGGCGGCGGCGGGGGCGCGGCGGGGACCGGC  
E E A R G S E A L G G G G G G A A G T R

110 130 150  
GAGGCGCGGACAAGGCGGTCCACGAGCGCGGTCACTGGAACAACAAGGTGGAGTTCGTG  
E A R D K A V H E R G H W N N K V E F V

170 190 210  
TTGAGCGTAGCGGGAGAGATCATCGGTCTGGGCAACGTGTGGCGCTTCCCCTACCTGTGC  
L S V A G E I I G L G N V W R F P Y L C

230 250 270  
TACAAGAACGGCGGAGGGGCATTCTGATTCTTACGTGGTGTTCATCTGCTGTGGA  
Y K N G G G A F L I P Y V V F F I C C G

290 310 330  
ATCCCCGTCTTCTTCTGGAACGGCTCTGGGGCAGTTCACGAGCGAGGGCGGCATCAGC  
I P V F F L E T A L G Q F T S E G G I T

350 370 390  
TGCTGGAGGAGAGTCTGTCCTTTATTTGAAGGCATCGGCTATGCAACACAGGTGATCGAG  
C W R R V C P L F E G I G Y A T Q V I E

410 430 450  
GCGCATCTCAATGTCTACTACATCATCCTGGCGTGGGCCATCTTCTACTTAAGCAAC  
A H L N V Y Y I I I L A W A I F Y L S N

470 490 510  
TGCTTACCACCGAGCTCCCCTGGGGCACCTGTGGGCATGAGTGGAACACAGAGAAATGT  
C F T T E L P W A T C G H E W N T E K C

530 550 570  
GTGGAGTTCCAGAAGCTGAACTTCAGCAACTACAGTCATGTGTCCCTGCAGAACGCAACC  
V E F Q K L N F S N Y S H V S L Q N A T

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590 610 630  
TCCCGGTCATGGAGTTCTGGGAACGCCGGGTCTTGGCTATATCTGATGGCATTGAACAC  
S P V M E F W E R R V L A I S D G I E H

650 670 690  
ATCGGGAACCTCCGATGGGAGCTGGCACTGTGTCTCCTGGCGGCTTGGACCATCTGCTAC  
I G N L R W E L A L C L L A A W T I C Y

710 730 750  
TTCTGCATCTGGAAGGGTACGAAGTCAACTGGAAAGGTCGTGTATGTCACCTGCAACCTTC  
F C I W K G T K S T G K V V Y V T A T F

770 790 810  
CCCTACATCATGCTGCTGATCCTCCTGATCCGAGGGGTACGTTGCCGGGTGCCTCGGAA  
P Y I M L L I L L I R G V T L P G A S E

830 850 870  
GGCATCAAGTTCTACCTGTACCCTGACCTCTCCCGGCTCTCTGATCCACAGGTGTGGGTG  
G I K F Y L Y P D L S R L S D P Q V W V

890 910 930  
GATGCTGGGACGCAGATCTTTTCTCCTATGCCATCTGCCTGGGCTGCCTGACCGCTCTG  
D A G T Q I F F S Y A I C L G C L T A L

950 970 990  
GGGAGTTACAACAACCTATAACAACAACCTGCTACAGGGACTGTATTATGCTCTGCTGTCTG  
G S Y N N Y N N N C Y R D C I M L C C L

1010 1030 1050  
AACAGTGGCACCAGCTTCGTGGCTGGGTTTGCTATCTTCTCAGTCCTGGGCTTCATGGCG  
N S G T S F V A G F A I F S V L G F M A

1070 1090 1110  
TACGAGCAGGGCGTGCCTATTGCTGAGGTGGCAGAATCAGGTCCTGGACTGGCTTTCATC  
Y E Q G V P I A E V A E S G P G L A F I

1130 1150 1170  
GCCTACCCCAAGGCTGTCACTATGATGCCCTGTCCCCATTGTGGGCCACCCTGTTCTTC  
A Y P K A V T M M P L S P L W A T L F F

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1190 1210 1230  
ATGATGCTCATCTTCCTGGGCCTGGACAGTCAGTTTGTGTGTGTGGAGAGCCTTGTGACA  
M M L I F L G L D S Q F V C V E S L V T

1250 1270 1290  
GCCGTGGTTGACATGTACCCCAAGGTCTTCCGGCGGGGCTACCGGCGAGAACTGCTCATC  
A V V D M Y P K V F R R G Y R R E L L I

1310 1330 1350  
CTGGCCCTGTCCATTGTCTCTTATTTCTAGGCCTGGTGATGCTGACAGAGGGAGGCATG  
L A L S I V S Y F L G L V M L T E G G M

1370 1390 1410  
TACATTTTCCAGCTTTTGGACTCATACGCCGCCAGTGGCATGTGCTTGTCTCTTCGTGGCC  
Y I F Q L F D S Y A A S G M C L L F V A

1430 1450 1470  
ATCTTTGAGTGTGTCTGCATCGGCTGGGTGTATGGAAGTAACAGGTTCTATGACAATATT  
I F E C V C I G W V Y G S N R F Y D N I

1490 1510 1530  
GAGGACATGATTGGATACCGGCCACTGTCACTCATCAAGTGGTGCTGGAAAGTTGTGACC  
E D M I G Y R P L S L I K W C W K V V T

1550 1570 1590  
CCTGGGATCTGTGCGGCATCTTCATCTTCTTTCTGGTCAAGTACAAGCCGCTCAAGTAC  
P G I C A G I F I F F L V K Y K P L K Y

1610 1630 1650  
AACAAATGTGTACACATATCCTGCTTGGGGCTACGGCATTGGCTGGCTCATGGCTCTGTCC  
N N V Y T Y P A W G Y G I G W L M A L S

1670 1690 1710  
TCCATGCTGTGCATCCCGCTCTGGATCTTCATCAAGCTGTGGAAGACAGAGGGCACCCCTG  
S M L C I P L W I F I K L W K T E G T L

1730 1750 1770  
CCCGAGAAATTACAGAAGTTGACAGTCCCCAGCGCTGATCTGAAAATGAGGGGCAAGCTT  
P E K L Q K L T V P S A D L K M R G K L

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1790

1810

1830

1850

GGGGCCAGCCACGGATGGTGACCGTTAATGACTGTGAGGCCAAGGTCAAAGGCGACGGT  
G A S P R M V T V N D C E A K V K G D G

1850

1870

1890

ACCATCTCTGCCATCACAGAGAAGGAGACGCACTTCTGATCCCCGCCAGCCACTTGGATG  
T I S A I T E K E T H F

1910

TGTCTCCAGCCTTCCTTC

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FIGURE 1C

-120 -100 -80  
GCCAAGCCGCGATCGCCGCAATCCCGCCAGCCTCGGGCCGGGCCATCCGCTGTGGGCT  
-60 -40 -20  
TAGCCACCCAGATGCAGAGCCAGTGCCACAGCCTCTTCAGAGGAGCCTCTCAAGCAAAAC  
0 20 40  
GAGGAGATGGCCACCAAGGAGAAGCTTCAATGTCTGAAAGACTTCCACAAAGACATCCTG  
M A T K E K L Q C L K D F H K D I L  
60 80 100  
AAGCCTTCTCCAGGGAAGAGCCCAGGCACGCGGCCTGAGGATGAGGCTGATGGGAAGCCC  
K P S P G K S P G T R P E D E A D G K P  
120 140 160  
CCTCAGAGGGAGAAGTGGTCCAGCAAGATCGACTTTGTGCTGTCTGTGGCCGGAGGCTTC  
P Q R E K W S S K I D F V L S V A G G F  
180 200 220  
GTGGGTTTGGGCAATGTCTGGCGTTTCCCGTACCTCTGCTACAAAATGGTGGAGGTGCA  
V G L G N V W R F P Y L C Y K N G G G A  
240 260 280  
TTCCTCATACCGTATTTTATTTTCCTGTTTGGGAGCGGCCTGCCTGTGTTTTTCCTGGAG  
F L I P Y F I F L F G S G L P V F F L E  
300 320 340  
GTCATCATAGGCCAGTACACCTCAGAAGGGGGCATCACCTGCTGGGAGAAGATCTGCCCC  
V I I G Q Y T S E G G I T C W E K I C P  
360 380 400  
TTGTTCTCTGGCATTGGCTACGCGTCCATCGTCATCGTGTCCCTCCTGAATGTGTACTAC  
L F S G I G Y A S I V I V S L L N V Y Y  
420 440 460  
ATCGTCATCCTGGCCTGGGCCACATACTACCTATTCCAGTCTTCCAGAAGGATCTTCCC  
I V I L A W A T Y Y L F Q S F Q K D L P

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480 500 520  
TGGGCCCCTGCAACCATAGCTGGAACACGCCACAGTGCATGGAGGACACCCTGCGTAGG  
W A H C N H S W N T P Q C M E D T L R R

540 560 580  
AACGAGAGTCACTGGGTCTCCCTTAGCGCCGCAACTTCACTTCGCCTGTGATCGAGTTC  
N E S H W V S L S A A N F T S P V I E F

600 620 640  
TGGGAGCGCAACGTGCTCAGCCTGTCTCCGGAATCGACCACCCAGGCAGTCTGAAATGG  
W E R N V L S L S S G I D H P G S L K W

660 680 700  
GACCTCGCGCTCTGCCTCCTCTTAGTCTGGCTCGTCTGTTTTTCTGCATCTGGAAGGGT  
D L A L C L L L V W L V C F F C I W K G

720 740 760  
GTTCGGTCCACAGGCAAGGTTGTCTACTTCACTGCTACTTTCCCGTTTGCCATGCTTCTG  
V R S T G K V V Y F T A T F P F A M L L

780 800 820  
GTGCTGCTGGTCCGTGGACTGACCCTGCCAGGTGCTGGTGAAGGCATCAAATTCTACCTG  
V L L V R G L T L P G A G E G I K F Y L

840 860 880  
TACCCTAACATCAGCCGCCTTGAGGACCCACAGGTGTGGATCGACGCTGGAACCTCAGATA  
Y P N I S R L E D P Q V W I D A G T Q I

900 920 940  
TTCTTTTCTACGCTATCTGCCTGGGGGCCATGACCTCACTGGGAAGCTATAACAAGTAC  
F F S Y A I C L G A M T S L G S Y N K Y

960 980 1000  
AAGTATAACTCGTACAGGGACTGTATGCTGCTGGGATGCCTGAACAGTGGTACCAGTTTT  
K Y N S Y R D C M L L G C L N S G T S F

1020 1040 1060  
GTGTCTGGCTTCGCAATTTTTTCCATCCTGGGCTTCATGGCACAAGAGCAAGGGGTGGAC  
V S G F A I F S I L G F M A Q E Q G V D

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1080 1100 1120  
ATTGCTGATGTGGCTGAGTCAGGTCCTGGCTTGGCCTTCATTGCCTACCCAAAAGCTGTG  
I A D V A E S G P G L A F I A Y P K A V

1140 1160 1180  
ACCATGATGCCGCTGCCACCTTTTGGTCCATTCTGTTTTTTATTATGCTCCTCTTGCTT  
T M M P L P T F W S I L F F I M L L L L

1200 1220 1240  
GGACTGGACAGCCAGTTTGTGAAGTCGAAGGACAGATCACATCCTTGTTGATCTTTAC  
G L D S Q F V E V E G Q I T S L V D L Y

1260 1280 1300  
CCGTCCTTCCTAAGGAAGGGTTATCGTCGGGAAATCTTCATTGCCATCGTGTGCAGCATC  
P S F L R K G Y R R E I F I A I V C S I

1320 1340 1360  
AGCTACCTGCTGGGGCTGACGATGGTGACGGAGGGTGGCATGTATGTGTTTCAACTCTTT  
S Y L L G L T M V T E G G M Y V F Q L F

1380 1400 1420  
GACTACTATGCAGCTAGTGGTGTATGCCTTTTGTGGGTGCGATTCTTTGAATGTTTGT  
D Y Y A A S G V C L L W V A F F E C F V

1440 1460 1480  
ATTGCCTGGATATATGGCGGTGATAACTTATATGACGGTATTGAGGACATGATCGGCTAT  
I A W I Y G G D N L Y D G I E D M I G Y

1500 1520 1540  
CGGCCTGGACCCTGGATGAAGTACAGCTGGGCTGTCTCATCTCCAGCTCTCTGTGTTGGA  
R P G P W M K Y S W A V I T P A L C V G

1560 1580 1600  
TGTTTCATCTTCTCTCTCGTCAAGTATGTACCCCTGACCTACAACAAAGTCTACCGGTAC  
C F I F S L V K Y V P L T Y N K V Y R Y

1620 1640 1660  
CCTGATTGGGCAATCGGGCTGGGCTGGGGCTGGCCCTTTCCTCCATGGTGTGTATCCQC  
P D W A I G L G W G L A L S S M V C I P

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1680 1700 1720  
TTGGTCATTGTCATCCTCCTCTGCCGGACGGAGGGACCGCTCCGCGTGAGAATCAAATAC  
L V I V I L L C R T E G P L R V R I K Y

1740 1760 1780  
CTGATAACCCCCAGGGAGCCCAACCGCTGGGCTGTGGAGCGTGAAGGGGCTACGCCCTTT  
L I T P R E P N R W A V E R E G A T P F

1800 1820 1840  
CACTCCAGAGCAACCCTCATGAACGGTGCACTCATGAAACCCAGTCACGTCATTGTGGAG  
H S R A T L M N G A L M K P S H V I V E

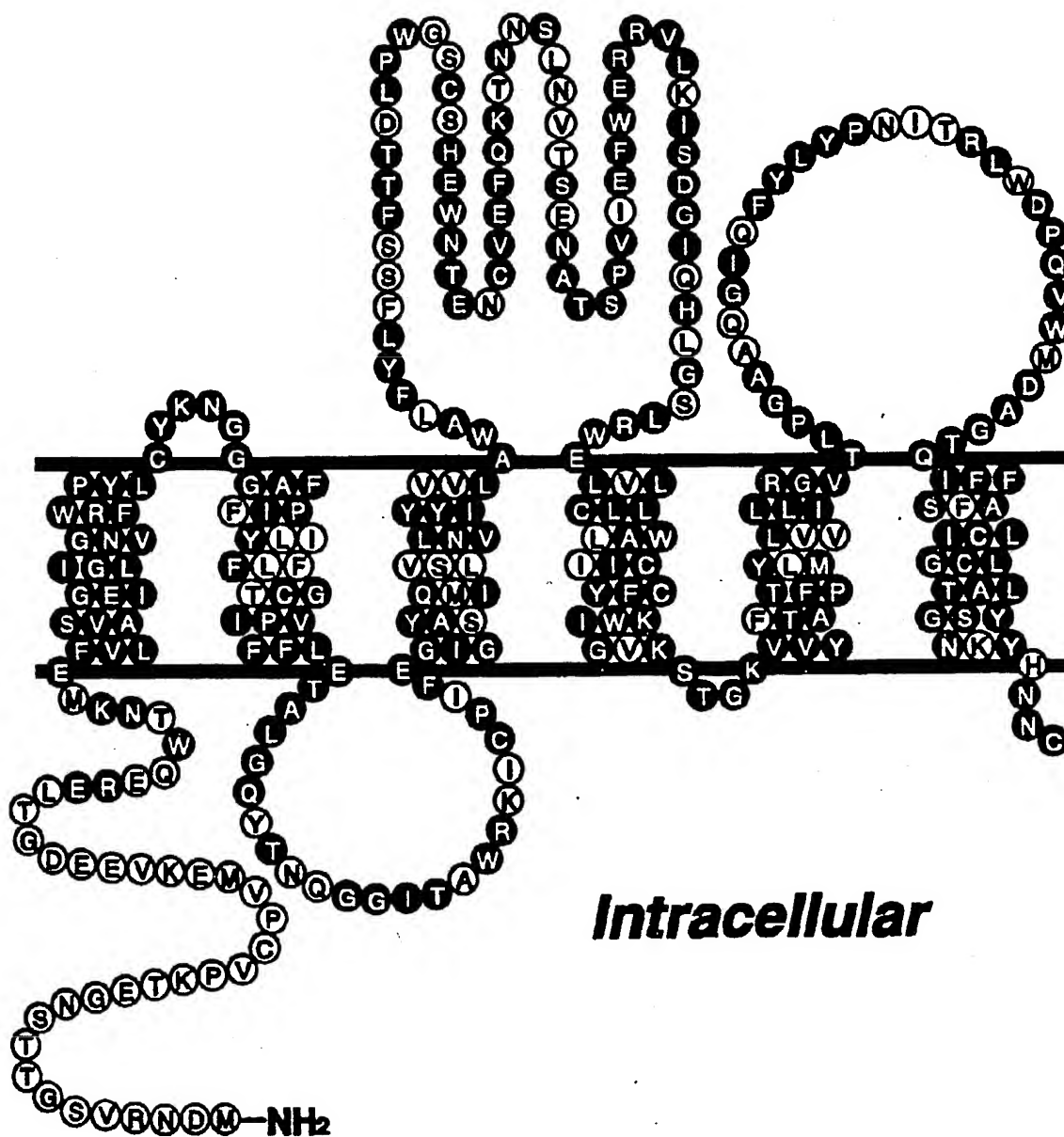
1860 1880 1900  
ACCATGATGTGAGGTCCGGGCTGTGTGACCGGCGCCGCTTTCCTGCCGTTTACTAACCTT  
T M M

1920 1940 1960  
AGATTCTCCTAGGACCAGGTTTACAGAGCTTTATATTTGTACTAGGATTTTTT

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FIGURE 1D

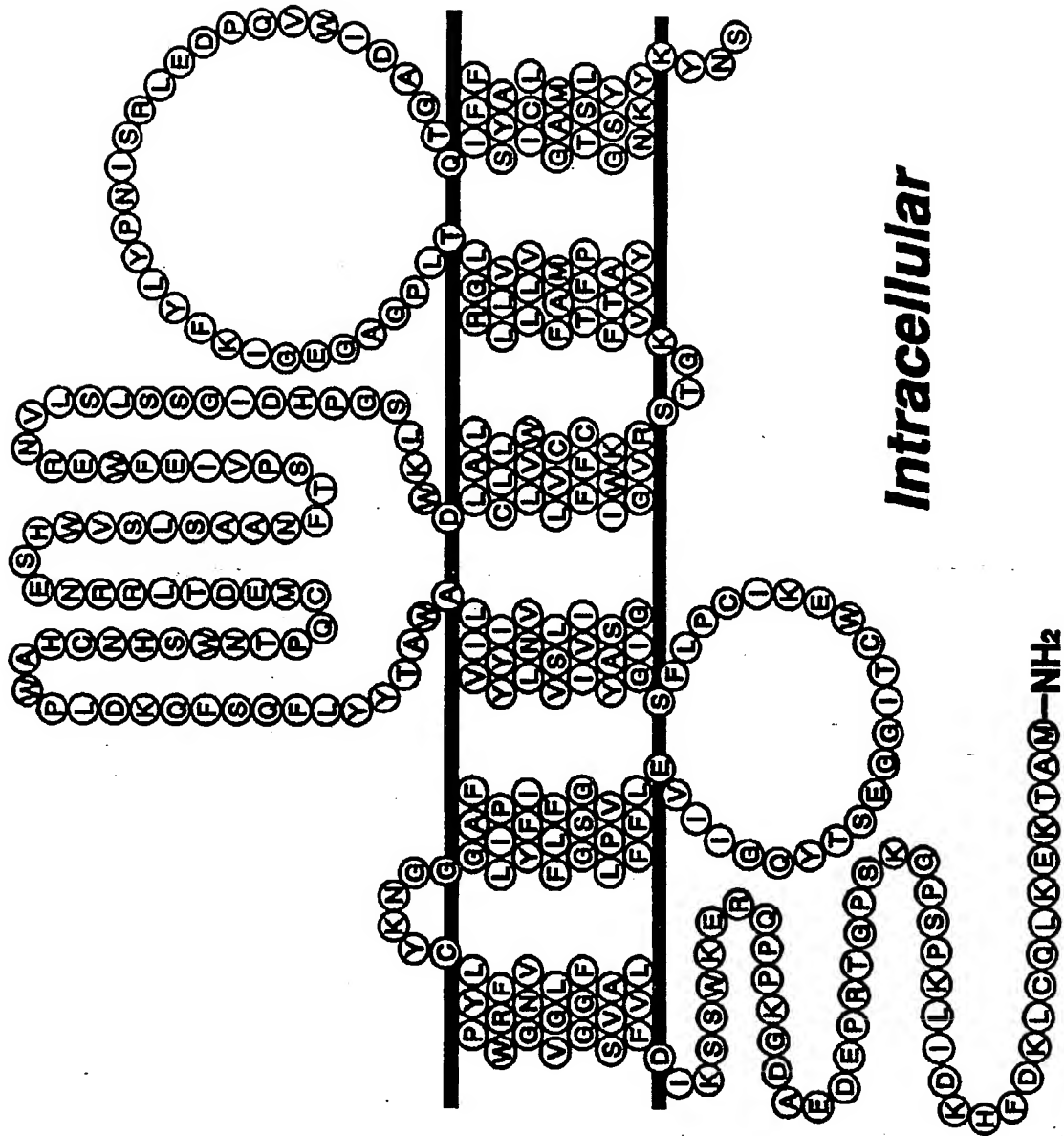


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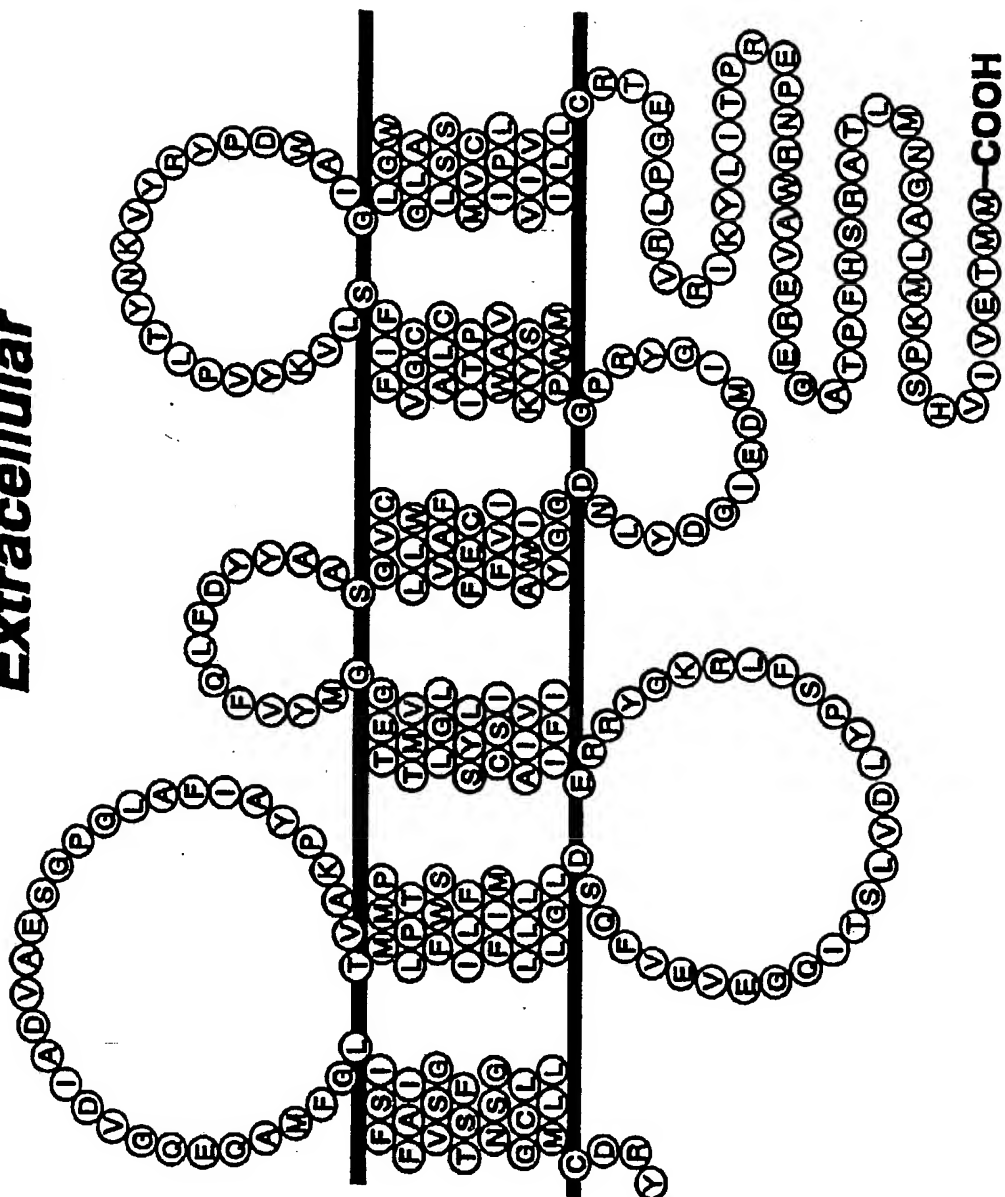




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FIGURE 1E



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**Extracellular**

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	177	192	183	182	187
GAT-2	.....	.....	.....	.....	.....
GAT-3	.....	.....	.....	.....	.....
GAT-1	.....	.....	.....	.....	.....
Betaine	.....	.....	.....	.....	.....
Glycine	.....	.....	.....	.....	.....

	233	238	239	248	257
GAT-2	.....	.....	.....	.....	.....
GAT-3	.....	.....	.....	.....	.....
GAT-1	.....	.....	.....	.....	.....
Betaine	.....	.....	.....	.....	.....
Glycine	.....	.....	.....	.....	.....

GAT-2  
GAT-3  
GAI-1  
Betaine  
Glycine

273  
288  
269  
279  
278  
287

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[illegible]

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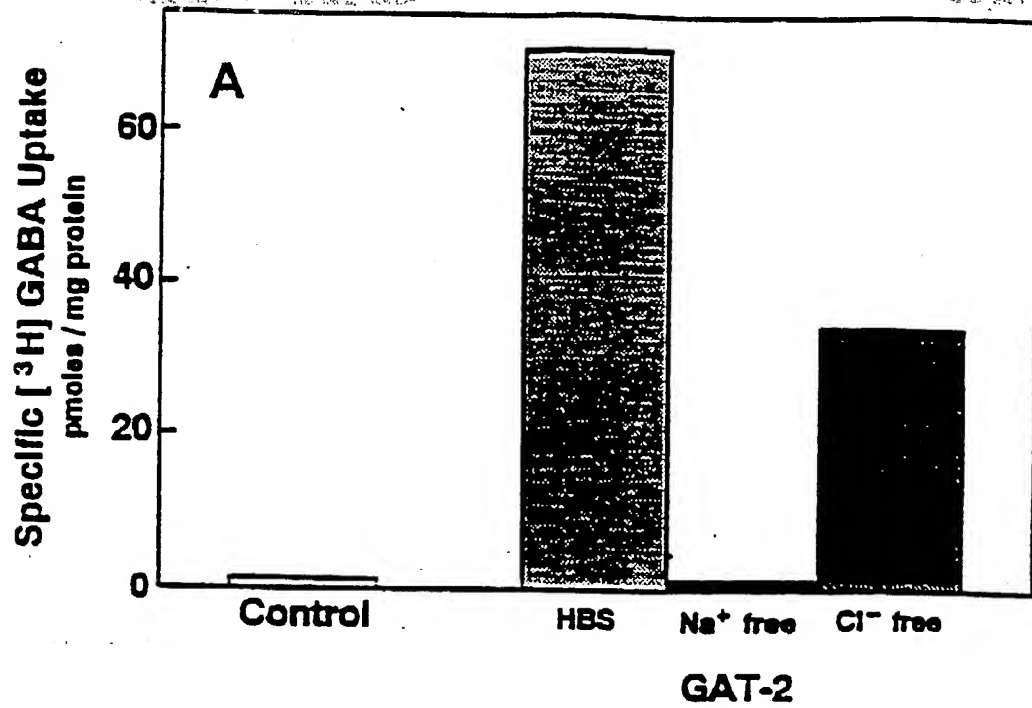
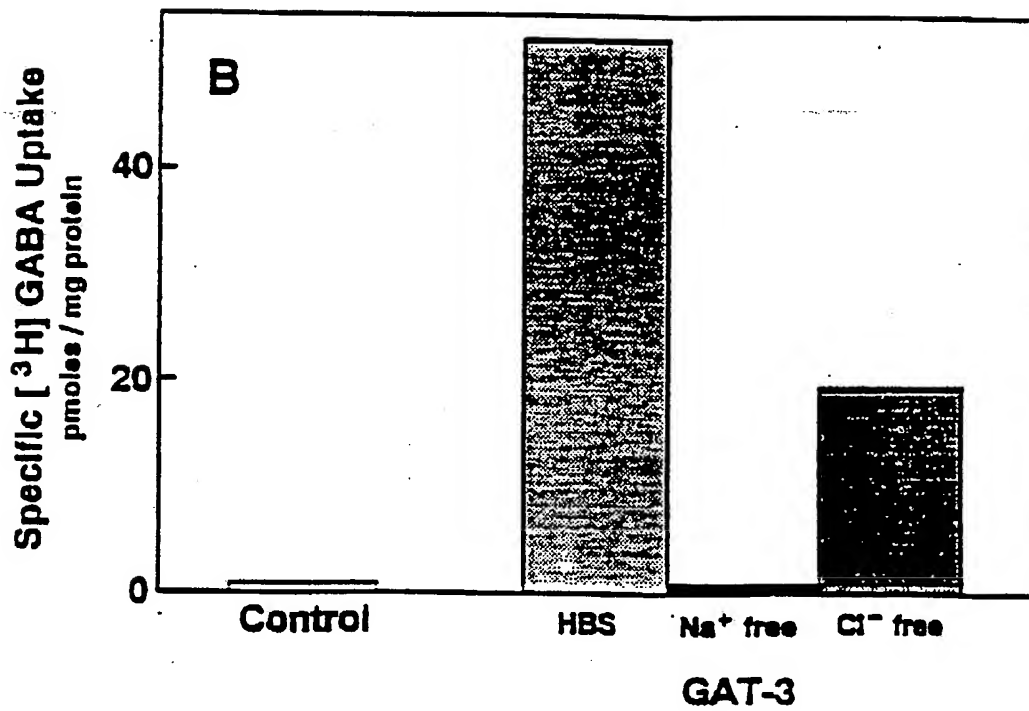
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FIGURE 3A

FIGURE 3B



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FIGURE 4A

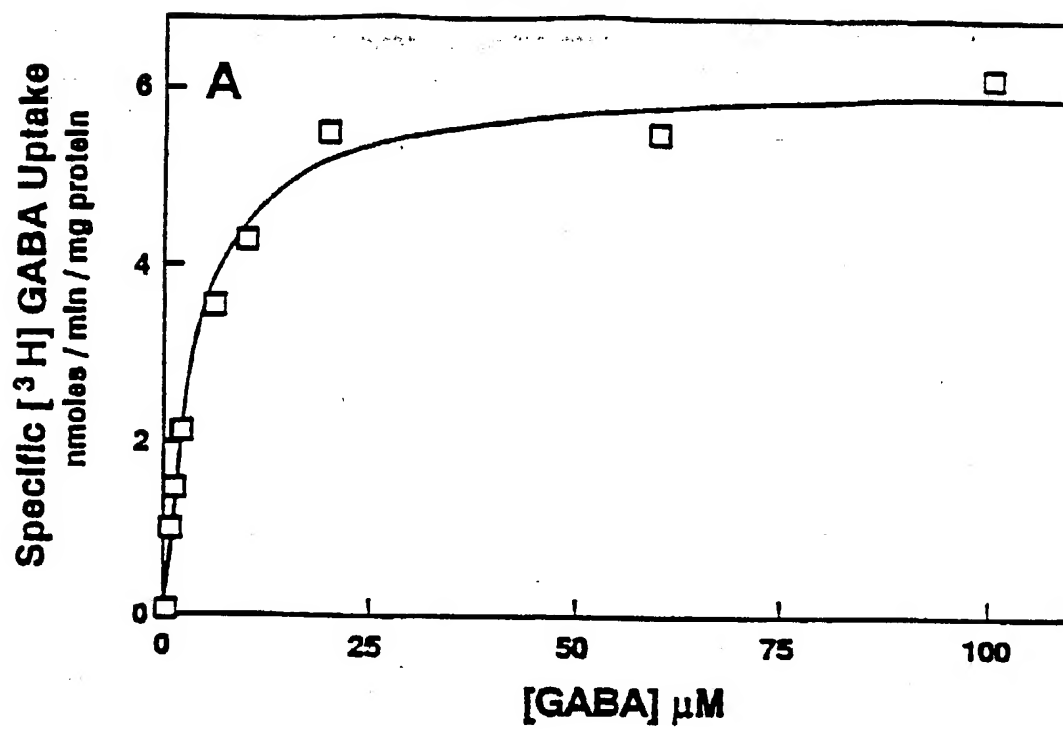
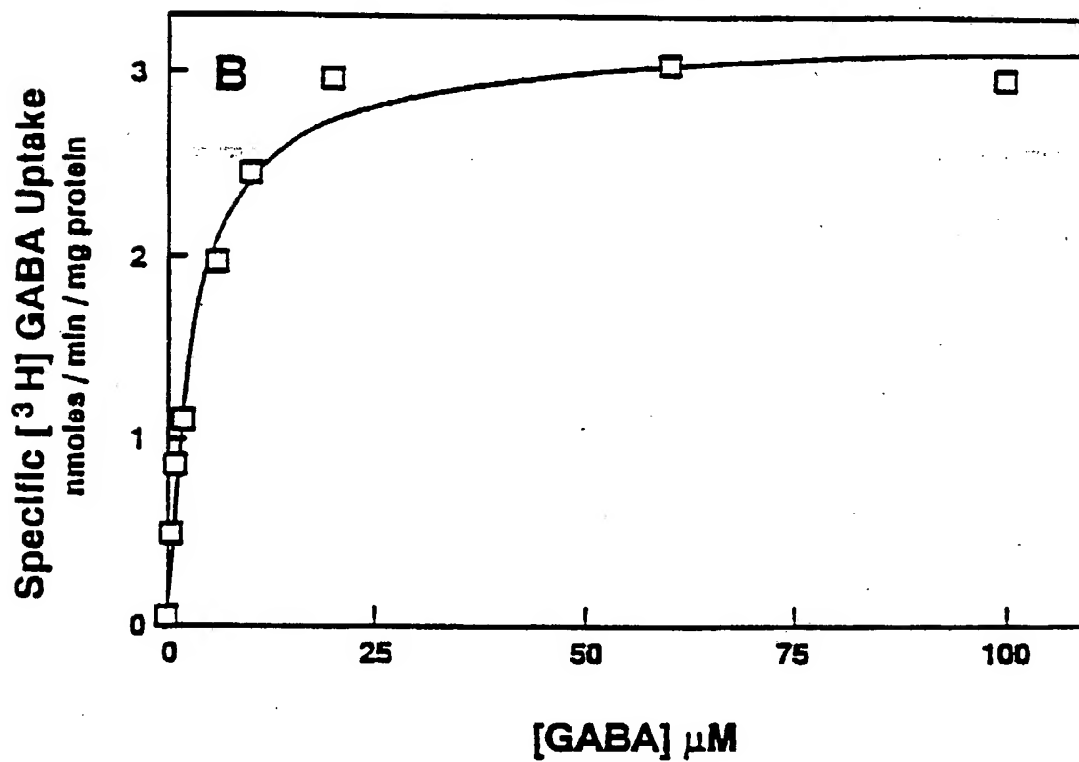


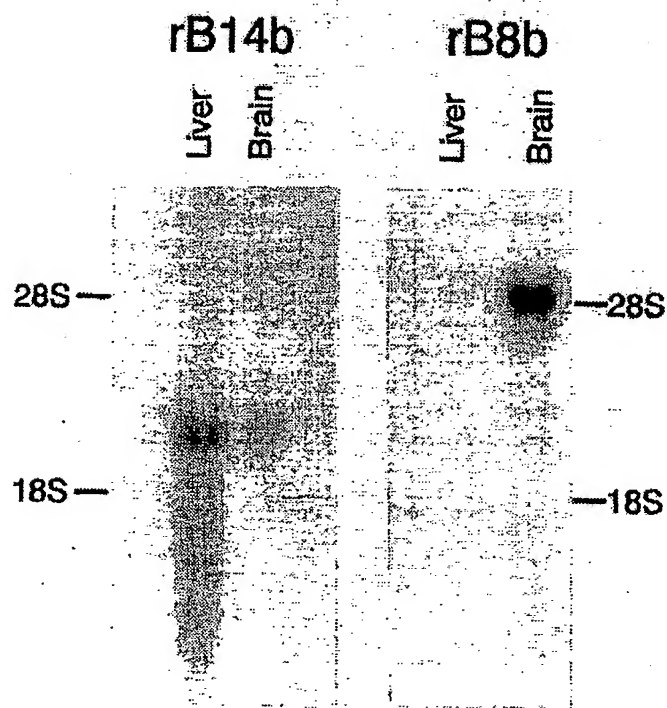
FIGURE 4B



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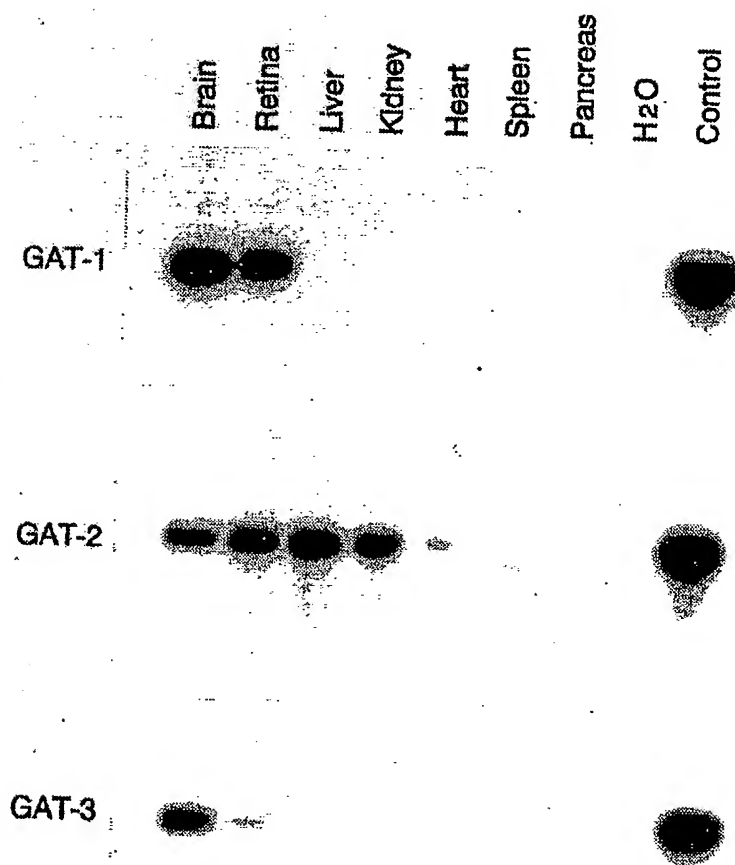
FIGURE 5A



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FIGURE 5B



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FIGURE 6

Taurine	M A T K E K L Q C L K D F H K D I L K P R P G G K S P G T R P . . . E D E A D G K P P Q R E K	43
GAT-1	M A T D N S K V A D G G I S T E V S E A P V A S D K P K T L V V K V Q K K A G D L P D R D T	46
Betaine	M A T D N S K V A D G G I S T E V S E A P V A S D K P K T L V V K V Q K K A G D L P D R D T	38
Glycine	M A T D N S K V A D G G I S T E V S E A P V A S D K P K T L V V K V Q K K A G D L P D R D T	34
Taurine	M A T K E K L Q C L K D F H K D I L K P R P G G K S P G T R P . . . E D E A D G K P P Q R E K	93
GAT-1	M A T D N S K V A D G G I S T E V S E A P V A S D K P K T L V V K V Q K K A G D L P D R D T	96
Betaine	M A T D N S K V A D G G I S T E V S E A P V A S D K P K T L V V K V Q K K A G D L P D R D T	88
Glycine	M A T D N S K V A D G G I S T E V S E A P V A S D K P K T L V V K V Q K K A G D L P D R D T	84
Taurine	M A T K E K L Q C L K D F H K D I L K P R P G G K S P G T R P . . . E D E A D G K P P Q R E K	143
GAT-1	M A T D N S K V A D G G I S T E V S E A P V A S D K P K T L V V K V Q K K A G D L P D R D T	145
Betaine	M A T D N S K V A D G G I S T E V S E A P V A S D K P K T L V V K V Q K K A G D L P D R D T	138
Glycine	M A T D N S K V A D G G I S T E V S E A P V A S D K P K T L V V K V Q K K A G D L P D R D T	133
Taurine	M A T K E K L Q C L K D F H K D I L K P R P G G K S P G T R P . . . E D E A D G K P P Q R E K	189
GAT-1	M A T D N S K V A D G G I S T E V S E A P V A S D K P K T L V V K V Q K K A G D L P D R D T	183
Betaine	M A T D N S K V A D G G I S T E V S E A P V A S D K P K T L V V K V Q K K A G D L P D R D T	182
Glycine	M A T D N S K V A D G G I S T E V S E A P V A S D K P K T L V V K V Q K K A G D L P D R D T	183

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	281	275	274	283
Taurine	F	F	F	F
GAT-1	Y	Y	Y	Y
Betaine	Y	Y	Y	Y
Glycine	F	F	F	F

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
Taurine	I	S	R	L	E	D	P	Q	V	V	I	D	A	G	T	T	V	P	P	S	T	A	L	G	L	G	A	N	S	L	G	S	T	N	K	Y	K	Y	X	Y	H	S	Y	P	D	C	M	L	G	C	331																																																	
GAT-1	F	R	K	L	S	D	S	E	V	V	L	D	A	A	T	T	T	P	P	S	T	A	L	G	L	G	S	L	Y	A	L	G	S	T	N	S	F	H	N	V	P	D	S	I	V	C	C	325																																																				
Betaine	L	L	R	L	K	D	P	Q	V	V	D	A	D	T	T	T	T	P	P	S	T	A	L	G	L	G	S	L	T	A	L	G	S	T	N	K	Y	K	Y	H	N	V	P	D	S	I	A	L	C	F	324																																																	
clycine	W	D	K	I	L	E	A	K	V	V	G	D	A	S	Q	T	P	P	S	T	A	L	G	L	G	A	N	S	L	G	S	T	N	K	Y	K	Y	H	N	V	P	D	S	I	A	L	C	F	323																																																			

[illegible]

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VIII  
Taurine P L P T F V S I E F F I N L P A L G G D G G F V E Y E G Q I T S L V D L Y P S F L R K G Y P R E I F 431  
GAT-1 P I S P L V A I L E F F S M L L M L G I D S Q F C T Y E G F I T A L V D E Y P R L L R N . . R R E L F 423  
Betaine P L S Q L V S C L E F F A T M L I F F L G E L D S Q F V C V E C L V T A S M D N F P S Q L R K S G R R E L L 424  
Glycine P I S P L V G L L C L F C F F M L I L L G L G T Q F C L L E T L V T A I V D E V G N E . W I L Q K K T Y V 432

IX  
Taurine T A I V C S I S Y L L L Q Q L T M V T L G G N Y Y F P Q L F F D Y T A A G G V C L L M V A F F E C F V I A W 481  
GAT-1 T A A V C I V S Y E I I G L S N I T Q G G I I Y V P K L F F D Y T A S A G G M S L L F L V P F E C V S I S W 473  
Betaine T L A I A V F C Y L A G L F L V T E G G N Y I F O L F D Y T A S S G I C L L F L A M F E V I C I S W 474  
Glycine Y L G V A V A G F L L Q I P L T S Q A G I Y W L L L L A D M Y A A G . F S L V V I S C I N C V S I M Y 481

XI  
Taurine I Y G G D N L Y D G T E D N I G Y R P P G D N M T Y R V A V I T P A L L C V G C F I F S L V K Y V P L T 531  
GAT-1 F Y G V N R F F D N I Q E M V G S R P C I I U K L C V S F F T P I I V A Q V F L F S A V Q M T P L T 523  
Betaine V Y G A D R F F D N I E D N I G Y R P P L V E I R V L F L T P G I C L L A T F L F S L S Q Y T P L K 524  
Glycine I Y G H R N Y F Q D I Q M N L Q F P P L F F Q I C H R F V S P I I Y F F I L I P T V I O Y R P I T 531

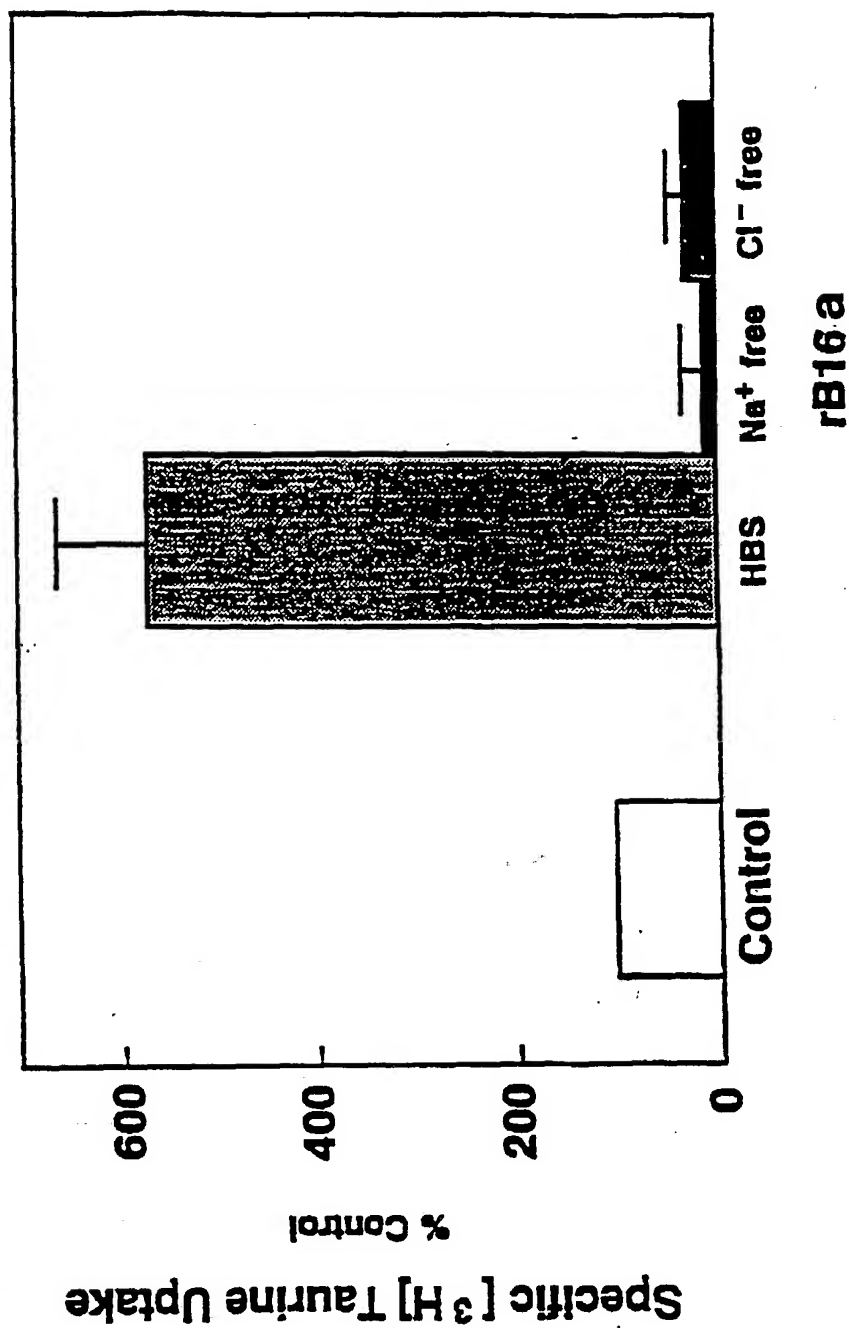
XII  
Taurine T R K V I R Y P D W A I I G L G G L A L S R N V G T P L V I V I L E G R T E G . P I R V R I K Y I I 580  
GAT-1 M G S . Y V F P K W G Q Q V G V L M A L S S N V L T P G Y M A Y M F L T L K G . S L K Q R L Q V M I 571  
Betaine Y N N I Y V Y P P W G Y S I G V F L A E P S M I C V P L F V I I T L L K T R G . S F K K R L R Q L T 573  
Glycine Y N H . T Q Y P G V A V A I G F L M A L S B V I C I P L Y A L F O L G R T D G D T L Q R L K N A T 580

Taurine T P R E P N R W A V E R E G A T P F R S R A I L M N G A L M K P S H V I V E T M . . . . . 621  
GAT-1 Q P S E D I V R P E N G P E Q P Q A G S S A S K E A Y I . . . . . 599  
Betaine T P D P S L P Q P K Q H L Y L D G G T S Q D C G P S P T K E G L I V G E K E T H L . . . . . 614  
Glycine K P S R D W G P A L L E H R T G R Y A P I T T P S P E D G F E V Q P L H P D K A Q I P I V G S N G S 630

Taurine . . . . . 621  
GAT-1 . . . . . 599  
Betaine . . . . . 614  
Glycine S R L Q D S R I 639

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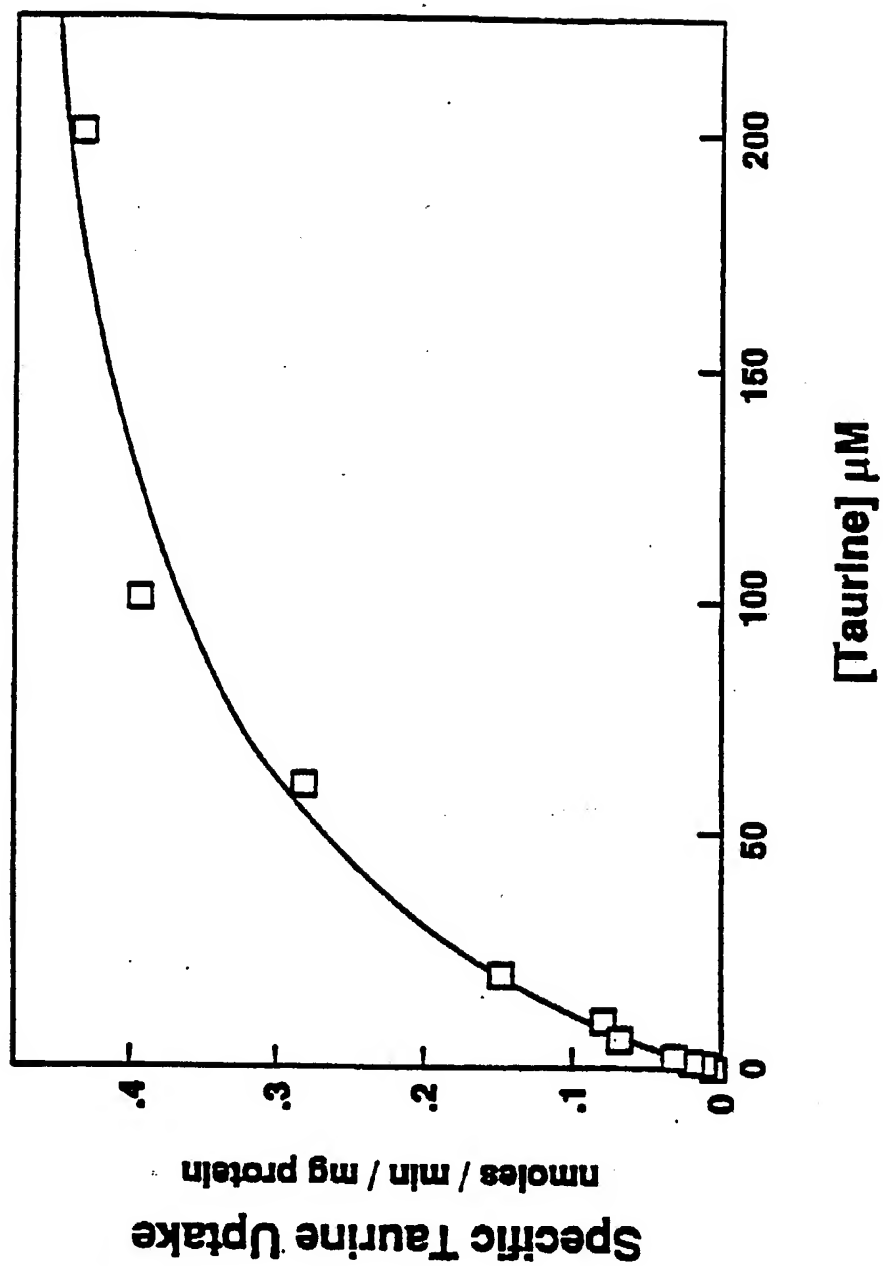
FIGURE 7



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FIGURE 8

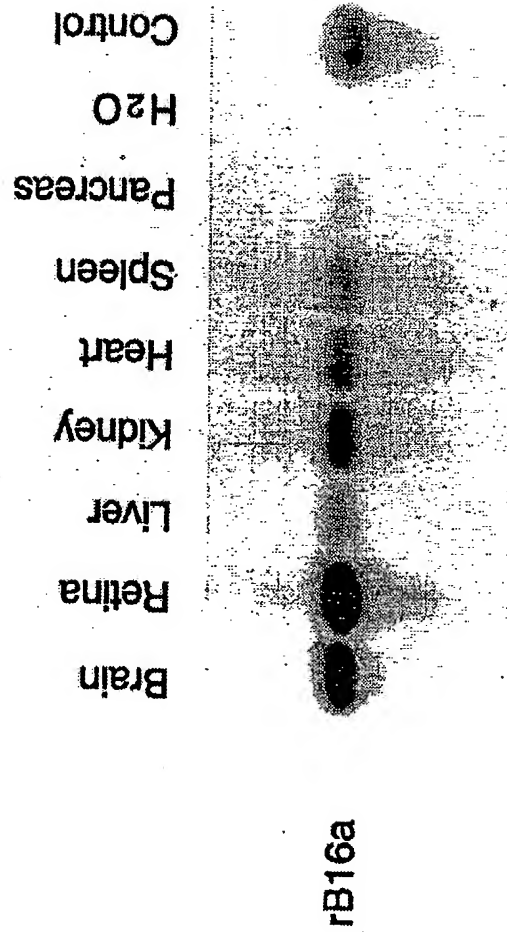


SUBSTITUTE SHEET



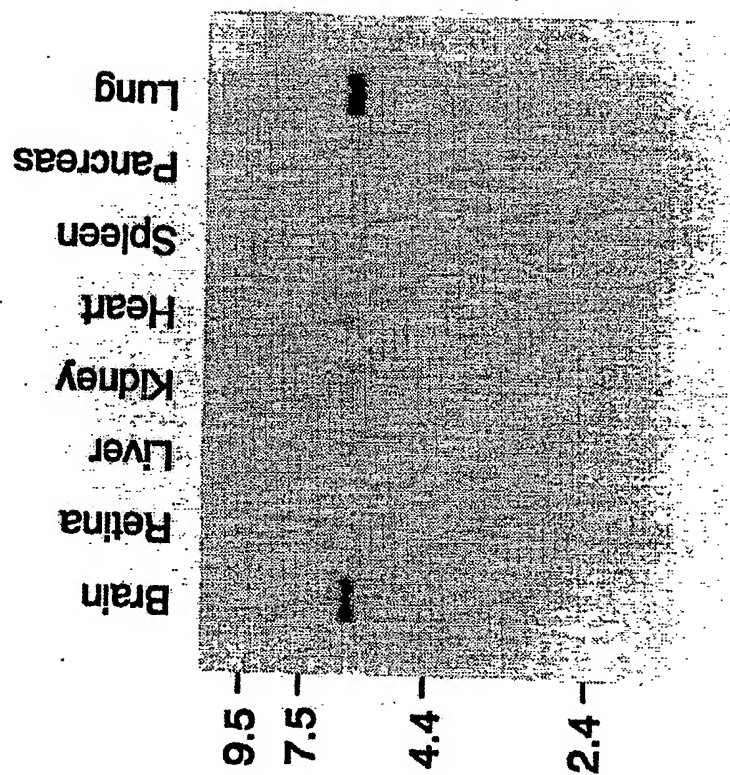
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FIGURE 9A



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FIGURE 9B



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FIGURE 10A

10 30 50  
CTGGCTTTCATCGCTTACCCGCGGGCTGTGGTGATGCTGCCCTTCTCTCTCTCTGGGGCC  
L A F I A Y P R A V V M L P F S P L W A

70 90 110  
TGCTGTTTCTTCTTCATGGTCGTTCTCCTGGGACTGGATAGCCAGTTTGTGTGTGTAGAA  
C C F F F M V V L L G L D S Q F V C V E

130 150 170  
AGCCTGGTGACAGCGCTGGTGGACATGTACCCTCACGTGTTCCGCAAGAAGAACCGGAGG  
S L V T A L V D M Y P H V F R K K N R R

190 210 230  
GAAGTCCTCATCCTTGGAGTATCTGTGCTCTCCTTCCTTGTGGGGCTGATCATGCTCACA  
E V L I L G V S V V S F L V G L I M L T

250 270 290  
GAGGGCGGAATGTACGTGTTCCAGCTCTTTGACTACTATGCGGCCAGTGGCATGTGCCTC  
E G G M Y V F Q L F D Y Y A A S G M C L

310 330 350  
CTGTTGCTGGCCATCTTCGAGTCCCTCTGTGTGGCTTGGGTTTACGGAGCCAAGCGCTTC  
L F V A I F E S L C V A W V Y G A K R F

370 390 410  
TACGACAACATCGAAGACATGATTGGGTACAGGCCATGGCCTCTTATCAAATACTGTTGG  
Y D N I E D M I G Y R P W P L I K Y C W

430 450 470  
CTCTTCCTCACCAGCTGTGTGCACAGCCACCTTTCTCTCTCCCTGATAAAGTACACT  
L F L T P A V C T A T F L F S L I K Y T

490 510 530  
CCGCTGACCTACAACAAGAAGTACACGTACCCGTGGTGGGGCGATGCCCTGGGCTGGCTC  
P L T Y N K K Y T Y P W W G D A L G W L

550 570 590  
CTGGCTCTGTCTCCATGGTCTGCATTCTGCCTGGAGCCTCTACAGACTCGGAACCCCTC  
L A L S S M V C I P A W S L Y R L G T L

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610 630 650  
AAGGGCCCCCTTCAGAGAGAGAATCCGTCAGCTCATGTGCCAGCCGAGGACCTGCCCCAG  
K G P F R E R I R Q L M C P A E D L P Q

670 690 710  
CGGAACCCAGCAGGACCCCTCGGCTCCCGCCACCCCCAGGACCTCACTGCTCAGACTCACA  
R N P A G P S A P A T P R T S L L R L T

730 750 770  
GAGCTAGAGTCTCACTGCTAGGGGGCAGGCCCTTGGATGGTGCCTGTGTGCCTGGCCTTG  
E L E S H C

790 810 830  
GGGATGGCTGTGGAGGGAACGTGGCAGAAGCAGCCCCATGTGCTTCCCTGCCCCCGACCT

850 870 890  
GGAGTGGATAAGACAAGAGGGGTATTTTGGAGTCCACCTGCTGAGCTGGAGGCCTCCAC

910 930 950  
TGCAACTTTTCAGCTCAGGGGTTGTTGAACAGATGTGAAAGGCCAGTGCCAAGAGTGTCC

970 990 1010  
CTCTGAGACCCTTGGGAAGCTGGGTGGGGGCTGGTAGGTGGGGCGAGACTGCTGGCTTC

1030 1050  
GGGCCCTCTCATCCTTCATTCCATTAAATCC

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FIGURE 10B

-30 -10 10  
AGCCGGGCGCGGCACGAGGCAGCCAGCGCGGCCATGACGGCGGAGAAGGCGCTGCCCCCT  
M T A E K A L P L

30 50 70  
GGGCAATGGGAAGGCTGCTGAGGAGGCGCGGGAGTCCGAGGCGCGGGTGGCGGCTGCAG  
G N G K A A E E A R E S E A P G G G C S

90 110 130  
CAGCGGGGCGCGGCGCGCCCGCGGCCACCCGCGCGTCAAGCGCGACAAGGCGGTCCACGA  
S G G A A P A R H P R V K R D K A V H E

150 170 190  
GCGCGGCCACTGGAACAACAAGGTGGAGTTCGTGCTGAGCGTGGCCGCGGAGATCATTGG  
R G H W N N K V E F V L S V A G E I I G

210 230 250  
GCTGGGCAACGTGTGGCGCTTCCCCTACCTGTGCTACAAGAACGGAGGAGGGGCATTCCCT  
L G N V W R F P Y L C Y K N G G G A F L

270 290 310  
GATTCCTACGTGGTGTTTTTTATTGCTGTGGAATTCCTGTTTTTCTGGAGACAGC  
I P Y V V F F I C C G I P V F F L E T A

330 350 370  
TCTGGGCGAGTTCACAAGTGAAGGTGGCATTACGTGTTGGAGGAAAGTTTGCCCTTTATT  
L G Q F T S E G G I T C W R K V C P L F

390 410 430  
TGAAGGCATTGGCTATGCAACACAGGTGATTGAGGCCCATCTGAATGTGTACTACATCAT  
E G I G Y A T Q V I E A H L N V Y Y I I

450 470 490  
CATCCTGGCATGGGCCATTTTTTACCTGAGCAACTGCTTCACTACTGAGCTACCCTGGGC  
I L A W A I F Y L S N C F T T E L P W A

510 530 550  
TACCTGTGGGCATGAGTGGAACACAGAGAATTGTGTGGAGTTCAGAACTGAATGTGAG  
T C G H E W N T E N C V E F Q K L N V S

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570 590 610  
CAA·CTACAGCCATGTGTCTCTGCAGAATGCCACCTCCCCTGTCATGGAGTTT·TGGGAGCA  
N Y S H V S L Q N A T S P V M E F W E H

630 650 670  
CCG·GGTCCTGGCCATCTCTGACGGGATCGAGCACATCGGGAACTTCGCTGGGAGCTGGC  
R V L A I S D G I E H I G N L R W E L A

690 710 730  
CTTGTGTCTCTTGGCAGCCTGGACCATCTGTTACTTCTGTATCTGGAAGGGGACCAAGTC  
L C L L A A W T I C Y F C I W K G T K S

750 770 790  
TACAGGAAAGGTTGTATACGTGACTGCGACATTCCCTACATCATGCTGCTGATCCTCCT  
T G K V V Y V T A T P P Y I M L L I L L

810 830 850  
GATACGAGGGGTCACGTTGCCCGGGGCTCAGAGGGCATCAAGTTCTACTTGTACCCTGA  
I R G V T L P G A S E G I K F Y L Y P D

870 890 910  
CCTCTCCCGGCTCTCCGACCCCCAGGTCTGGGTAGATGCTGGAACGCAGATCTTTTCTC  
L S R L S D P Q V W V D A G T Q I F F S

930 950 970  
CTATGCCATTTGCCTGGGCTGTCTGACCGCTCTGGGAAGTTATAACAATTATAACAACAA  
Y A I C L G C L T A L G S Y N N Y N N N

990 1010 1030  
CTGCTACAGGGACTGCATCATGCTCTGTTGCCTGAACAGCGGCACCAGCTTCGTGGCTGG  
C Y R D C I M L C C L N S G T S F V A G

1050 1070 1090  
GTTTGCCATCTTCTCAGTCCTGGGTTTATGGCGTACGAGCAGGGGGTACCCATTGCTGA  
F A I F S V L G F M A Y E Q G V P I A E

1110 1130 1150  
GGTGGCAGAGTCAGGCCCGGCCTGGCCTTTATTGCGTACCCCAAGGCGGTACCATGAT  
V A E S G P G L A F I A Y P K A V T M H

1170 1190 1210  
GCC·TCTCTCCCGCTGTGGGCCACCTTGTTCATGATGCTCATCTTCCTGGGCCTGGA

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F L S P L W A T L F F M M L I F L G L D  
1230 1250 1270  
CAGCCAGTTTGTGTGTGTGGAAAGCCTGGTGACCGCCGTGGTGGACATGTACCCCAAGGT  
S Q F V C V E S L V T A V V D M Y P K V  
1290 1310 1330  
TTTCCGGAGGGGTTACCGGCGGGAGCTGCTCATCCTAGCCTTGTCTGTTATCTCCTATTT  
F R R G Y R R E L L I L A L S V I S Y F  
1350 1370 1390  
TCTGGGCCTCGTGATGTTAACAGAGGGTGGCATGTACATCTTCCAGCTCTTTGACTCCTA  
L G L V M L T E G G M Y I F Q L F D S Y  
1410 1430 1450  
TGCCGCCAGTGGGATGTGCCTTCTCTTCGTGGCCATGTTTGAGTGCATCTGCATCGGCTG  
A A S G M C L L F V A I F E C I C I G W  
1470 1490 1510  
GGTGTATGGAAGCAACCGGTTCTATGATAACATTGAAGACATGATTGGCTACCGGCCACC  
V Y G S N R F Y D N I E D M I G Y R P P  
1530 1550 1570  
GTCGCTCATTAAAGTGGTGCTGGATGATCATGACCCCTGGGATCTGCGCGGGGATCTTCAT  
S L I K W C W M I M T P G I C A G I F I  
1590 1610 1630  
CTTCTTCTTGATCAAGTACAAGCCACTCAAGTACAACAACATCTACACCTACCCAGCCTG  
F F L I K Y K P L K Y N N I Y T Y P A W  
1650 1670 1690  
GGGCTATGGCATTGGCTGGCTCATGGCCCTGTCCTCCATGCTCTGCATCCCGCTCTGGAT  
G Y G I G W L M A L S S M L C I P L W I  
1710 1730 1750  
CTGCATCACAGTGTGGAAGACGGAGGGGACACTGCCCCAGAACTCCAGAAGTTGACGAC  
C I T V W K T E G T L P E K L Q K L T T  
1770 1790 1810  
CCCCAGCACAGATCTGAAAATGCGGGGCAAGCTTGGGGTGAGCCCACGGATGGTGACAGT  
P S T D L K M R G K L G V S P R M V T V  
1830 1850 1870

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TAATGACTGTGATGCCAACTCAAGAGTGACGGGACCATCGCAGCCATCACAGAGAAGGA  
N D C D A K L K S D G T I A A I T E K E

1890

1910

1930

GACGCACTTCTGAGCGGCCACCAGCCATCTGGGGCTCTTCTTCCTTTCTTCCCCCGTGT  
T H F \*

1950

ATGTAAATGAA

SUBSTITUTE SHEET



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/01959

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) : C12N 15/00, 15/12

US CL : 536/23.5; 435/6, 7.2, 69.1, 240.2, 255, 252.3, 320.1

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5; 435/6, 7.2, 69.1, 240.2, 255, 252.3, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS and DIALOG (files 5, 155, 351, 357,358), search terms: GABA,  $\gamma$ -aminobutyric acid, taurine, transporter, Cos7, antisense, ribozyme, channel**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P Y	Society for Neuroscience Abstracts, Volume 18, Part 1, issued 25-30 October 1992, K.E. Smith et al., "Cloning and Expression of a Taurine Transporter from Rat Brain," see page 473, abstract no. 202.3.	1 - 2 , 5 - 6 , 9 - 1 2 , 1 5 , 2 3 - 24,30,33,41,43.4 <u>5-51</u> 3-4,7-8,28-29,36

☒ Further documents are listed in the continuation of Box C.
 ☒ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be part of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A*	document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means		
*P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

03 JUNE 1993

Date of mailing of the international search report

17 JUN 1993

 Name and mailing address of the ISA/US  
 Commissioner of Patents and Trademarks  
 Box PCT  
 Washington, D.C. 20231

Facsimile No. NOT APPLICABLE

Authorized officer

MARIANNE PORTA ALLEN

Telephone No. (703) 308-0196

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US93/01959

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
<u>X, P</u> Y	Society for Neuroscience Abstracts, Volume 18, Part 1, issued 25-30 October 1992, L.A. Borden et al., "Cloning and Expression of Two Novel GABA Transporters from Rat Brain," see page 581, abstract no. 251.4.	1-2,9-10, 21,22,27,32, 40,42,44, <u>46-51</u> 3-4,25,26, 34,35,37-39
<u>X</u> Y	FEBS LETTERS, Volume 269, Number 1, issued 20 August 1990, H. Nelson et al., "Cloning of the human brain GABA transporter," pages 181-184, see entire document.	1-3,9-10,21- <u>22,46-51</u> 4, 25-27,32
A	TRENDS PHARMACOL. SCI., Volume 11, Number 11, issued November 1990, N.G. Bowery, "GABA transporter protein cloned from rat brain," pages 29-39, see entire document.	1-15,21-30,32- 60,68-75,115
<u>X, P</u> Y	JOURNAL OF BIOLOGICAL CHEMISTRY, Volume 267, Number 31, issued 05 November 1992, M.P. Kavanaugh et al., "Electrogenic Uptake of $\gamma$ -Aminobutyric Acid by a Cloned Transporter Expressed in <i>Xenopus</i> Oocytes," pages 22007-22009, see entire document.	1-2,9-10,21 <u>22,46-51</u> 3-4,25-27,32
<u>X, P</u> Y	JOURNAL OF BIOLOGICAL CHEMISTRY, Volume 267, Number 29, issued 15 October 1992, L.A. Borden et al., "Molecular Heterogeneity of the $\gamma$ -Aminobutyric Acid (GABA) Transport System," pages 21098-21104, see entire document.	1-2,9-10,21- 22,27,32,40, <u>42,44,46-51</u> 3-4,25-26, 34,37- 39
<u>X, P</u> Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, Volume 89, Number 17, issued 01 September 1992, S. Uchida et al., "Molecular cloning of the cDNA for an MDCK cell Na <sup>+</sup> - and Cl <sup>-</sup> -dependent taurine transporter that is regulated by hypertonicity," pages 8230-8234, see entire document.	5,11-12, 15,23- <u>24,46-51</u> 6-8
<u>X, P</u> Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, Volume 89, Number 24, issued 15 December 1992, Q.-R. Lui et al., "Cloning and expression of a cDNA encoding the transporter of taurine and $\beta$ -alanine in the mouse brain," pages 12145-12149, see entire document.	5,8,11-12, 15,23- <u>24,46-51</u> 6-7
<u>X, P</u> Y	JOURNAL OF BIOLOGICAL CHEMISTRY, Volume 267, Number 27, issued 05 September 1992, B. Lopez-Corcuera et al., "Expression of a Mouse Brain cDNA Encoding Novel $\gamma$ -Aminobutyric Acid Transporter," pages 17491-17493, see entire document.	1,4,9-10,21- 22,27,32, <u>46-51</u> 2-3,25-27,34,37- 39,40,42,44

## INTERNATIONAL SEARCH REPORT

 International application No.  
 PCT/US93/01959

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	BIOCHEMISTRY, Volume 31, Number 7, issued 25 February 1992, S. Keynan et al., "Expression of a Cloned $\gamma$ -Aminobutyric Acid Transporter in Mammalian Cells," pages 1974-1979, see entire document.	1-2,9-10,21-22, 27,32,40, <u>42,44,46-51</u> 3-4,25-26, 34,37-39
X Y	FEBS LETTERS, Volume 295, Number 1, 2, 3, issued December 1991, W. Mayser et al., "Isolation of cDNAs encoding a novel member of the neurotransmitter transporter gene family," pages 203-206, see entire document.	1-2,9-10, <u>21-22,46-51</u> 3-4,25-27
A	TINS, Volume 13, Number 12, issued 1990, M.J. Kuhar, "A GABA transporter cDNA has been cloned," pages 473-474, see entire document.	1-15,21-30,32-60,68-75,115
X Y	SCIENCE, Volume 249, issued 14 September 1990, J. Guastella et al., "Cloning and Expression of a Rat Brain GABA Transporter," pages 1303-1306, see entire document.	1-2,9-10, <u>21-22,46-51</u> 3-4,25-27
Y	JOURNAL OF NEUROCHEMISTRY, Volume 56, Number 3, issued March 1991, R.D. Blakely et al., "Distinct, Developmentally Regulated Brain mRNAs Direct the Synthesis of Neurotransmitter Transporters," pages 860-871, see entire document.	1-4,9-10,21-22,25-27,46-51
X, P Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, Volume 89, issued July 1992, Q.-R. Liu et al., "A family of genes encoding neurotransmitter transporters," pages 6639-6643, see entire document.	1, 4,9-10, 21-22, <u>46-51</u> 2-3
Y	BIOTECHNIQUES, Volume 6, Number 10, issued 1988, A.R. van der Krol et al., "Modulation of Eukaryotic Gene Expression by Complementary RNA or DNA Sequences," pages 958-973, see entire document.	46-60,68-75
X, P	Society for Neuroscience Abstracts, Volume 15, Pat 1, issued 25 October 1992, J. Guastella et al., "Expression of GABA-transporter mRNA in <i>Xenopus</i> Oocytes," see page 601, abstract no 242.8.	46-51
A, P	TRENDS IN NEUROSCIENCES, Volume 15, Number 7, issued July 1992, G.R. Uhl, "Neurotransmitter transporters (plus): a promising new gene family," pages 265-268, see entire document.	1-15,21-30,32-60,68-75,115

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US93/01959**Box I - Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 31, 76-79, 82, 145-147  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
  
Please See Extra Sheet.
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II - Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**This International Searching Authority found multiple inventions in this international application, as follows:  
(Form PCT/ISA/206 Previously Mailed.)  
Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:  
1-15, 21-30, 32-60, 68-75, 115
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐  
☒

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

**BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE****2. Where no meaningful search could be carried out, specifically:**

Claims 31 and 82 could not be searched because no claim 31 or 82 is present. Claims 76-79 could not be searched because the identity of the substances with the requisite properties cannot be determined from the specification. Claim 145 could not be searched because it cannot be determined what substance is being administered in the method of treatment. Claims 146-147 could not be searched because it cannot be determined what DNA would be examined for unidentified and unknown diseases. The vagueness of claims 76-79 and 145-147, even when read in light of the specification, does not permit a meaningful search.

**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING**

This ISA found multiple inventions as follows:

Group I, claims 1-4, 9-10, 13, 21-22, 25-27, 32, 34-35, 37-39, 40, 42, 44, 46-47, 50, 52-53, 57, 59, 68, 70-75, and 115, drawn to nucleic acid encoding GABA transporter, vector, transformed host cells, nucleic acid probes, antisense oligonucleotides, oligonucleotide pharmaceuticals, and a method of detecting expression of the GABA transporter.

Group II, claims 5-8, 11-12, 14-15, 23-24, 28-30, 33, 36, 41, 43, 45, 48-49, 51, 54-56, 58, 60, and 69, drawn to nucleic acid encoding taurine transporter, vector, transformed host cells, nucleic acid probes, antisense oligonucleotides, oligonucleotide pharmaceuticals, and a method for isolating a nucleic acid molecule encoding taurine receptor.

Group III, claim 148-149, drawn to a recombinant method of making the taurine transporter.

Group IV, claims 16-17, drawn to a GABA transporter protein.

Group V, claims 18-20, drawn to a taurine transporter protein.

Group VI, claims 61-62, 66, and 80, drawn to a monoclonal antibody to GABA transporter and pharmaceutical compositions containing antibody.

Group VII, claims 63-65, 67, and 81, drawn to a monoclonal antibody to taurine transporter and a pharmaceutical compositions containing antibody.

Group VIII, claim 76, drawn to a substance to alleviate abnormalities of overexpression of GABA transporter.

Group IX, claim 77, drawn to a substance to alleviate abnormalities of overexpression of taurine transporter.

Group X, claim 78, drawn to a substance to alleviate abnormalities of underexpression of GABA transporter.

Group XI, claim 79, drawn to a substance to alleviate abnormalities of overexpression of taurine transporter.

Group XII, claims 83, 85, 87, 89, 91, 95, and 97, drawn to transgenic animal with GABA transporter gene.

Group XIII, claims 84, 86, 88, 90, 92, 96, and 98, drawn to a transgenic animal with taurine transporter gene.

Group XIV, claims 101-102, 104, and 106, drawn to a method for determining substrates that bind to taurine transporter.

Group XV, claims 106 and 114, drawn to substrates that bind to GABA transporter.

Group XVI, claims 107 and 114, drawn to substrates that bind to taurine transporter.

Group XVII, claims 108, 110, and 112, drawn to a method of screening drugs that interact with GABA transporter using a plurality of drugs.

Group XVIII, claims 109, 111, and 113, drawn to a method of screening drugs that interact with taurine transporter using a plurality of drugs.

Group XIX, claims 148-149, drawn to a recombinant method of producing the GABA transporter.

Group XX, claim 116, drawn to a method of detecting expression of cell-surface taurine transporter using

**oligonucleotides.**

Group XXI, claims 117, 119, and 121, drawn to a method of treating patient with overexpression of GABA transporter using oligonucleotides.

Group XXII, claims 118 and 120-124, drawn to a method of treating patient with overexpression of taurine transporter using oligonucleotides.

Group XXIII, claims 125, 127, and 129-130, drawn to a method of treating patient with overexpression of GABA transporter using monoclonal antibodies.

Group XXIV, claims 126, 128-129, and 131-132, drawn to a method of treating patient with overexpression of taurine transporter using monoclonal antibodies.

Group XXV, claim 133, drawn to a method of detecting presence of cell-surface GABA transporter using antibodies.

Group XXVI, claim 134, drawn to a method of detecting presence of cell-surface taurine transporter using antibodies.

Group XXVII, claim 135, drawn to a method of determining varying levels of physiological effects of expressing varying levels of GABA transporter in a transgenic animal using an inducible promoter.

Group XXVIII, claim 136, drawn to a method of determining varying levels of physiological effects of expressing varying levels of taurine transporter in a transgenic animal using an inducible promoter.

Group XXIX, claim 137, drawn to a method of determining varying levels of physiological effects of expressing varying levels of GABA transporter in a transgenic animal using panels of transgenic animals.

Group XXX, claim 138, drawn to a method of determining varying levels of physiological effects of expressing varying levels of taurine transporter in a transgenic animal panels of transgenic animals.

Group XXXI, claim 139, drawn to a method of identifying substances alleviating effects of overexpression of GABA transporter using transgenic animal.

Group XXXII, claim 140, drawn to a method of identifying substances alleviating effects of overexpression of taurine transporter using transgenic animal.

Group XXXIII, claim 141, drawn to a method of treating subject with overexpression of GABA transporter by administering transgenic animal.

Group XXXIV, claim 142, drawn to a method of treating subject with overexpression of taurine transporter by administering transgenic animal.

Group XXXV, claim 143, drawn to a method of identifying substances alleviating effects of underexpression of GABA transporter using transgenic animal.

Group XXXVI, claim 144, drawn to a method of identifying substances alleviating effects of underexpression of taurine transporter using transgenic animal.

Group XXXVII, claim 145, drawn to a method of treating subject with underexpression of mammalian transporter by administering substance.

Group XXXVIII, claims 146-147, drawn to a method of diagnosing a predisposition associated with expression of a mammalian transporter.

Group XXXIX, claim 150, drawn to a method for preparing membranes containing GABA transporter.

Group XXXX, claim 151, drawn to a method for preparing membranes containing taurine transporter.

Group XXXXI, claim 152, drawn to a method for isolating vesicles comprising GABA transporter.

Group XXXXII, claim 153, drawn to a method for isolating vesicles comprising taurine transporter.

Group XXXXIII, claim 154, drawn to a method for identifying compound binding to GABA transporter using isolated membranes.

Group XXXXIV, claim 155, drawn to a method for identifying compound binding to taurine transporter using isolated membranes.

Group XXXXV, claim 156, drawn to a method for identifying compound binding to GABA transporter using isolated vesicles.

Group XXXXVI, claim 157, drawn to a method for identifying compound binding to taurine transporter using isolated vesicles.

Group XXXXVII, claim 158, drawn to a method for identifying compound which enhances or decreases GABA transporter activity using membrane vesicles.

Group XXXXVIII, claim 159, drawn to a method for identifying compound which enhances or decreases taurine transporter activity using membrane vesicles.

Group XXXXIX, claims 99-100, 103 and 105, drawn to a method for determining substrates that bind to GABA transporter.

It is noted that there is no claim 31 or 82 present in the application.

The inventions listed as Groups I through XXXXIX do not meet the requirements for Unity of Invention for the reasons that follow.

Group I forms a first single general inventive concept of a first appearing product, and a first appearing use of the said product for the nucleic acids encoding the GABA transporter.

Group II forms a second single general inventive concept of a second appearing product, and a first appearing use of the said product for the nucleic acids encoding the taurine transporter.

Groups IV, VI, VIII, X, XII, and XV are drawn to additional compositions associated with the GABA transporter. The protein, monoclonal antibody, unidentified substances, transgenic animal, and unidentified substrate are distinct compositions that do not rely upon each other.

Groups V, VII, IX, XI, XIII, and XVI are drawn to additional compositions associated with the taurine transporter. The protein, monoclonal antibody, unidentified substances, transgenic animal, and unidentified substrate are distinct compositions that do not rely upon each other.

Groups XVII, XIX, XXI, XXIII, XXV, XXVII, XXIX, XXXI, XXXIII, XXXV, XXXXIX, XXXXI, XXXXIII, XXXXV, XXXXVII, and XXXXXIX are drawn to additional methods with different goals and method steps. These methods are associated with the GABA transporter.

Groups III, XIV, XX, XXII, XXIV, XXVI, XXVIII, XXX, XXXII, XXXIV, XXXVI, XXXX, XXXXII, XXXXIV, XXXXVI, and XXXXVIII are drawn to additional methods with different goals and method steps. These methods are associated with the taurine transporter.

Groups XXXVII and XXXVIII are drawn to additional methods with different goals and method steps. These methods are associated with any mammalian transporter.

It is noted that the GABA transporter and taurine transporter are independent neurotransmitter transporters.

The claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.